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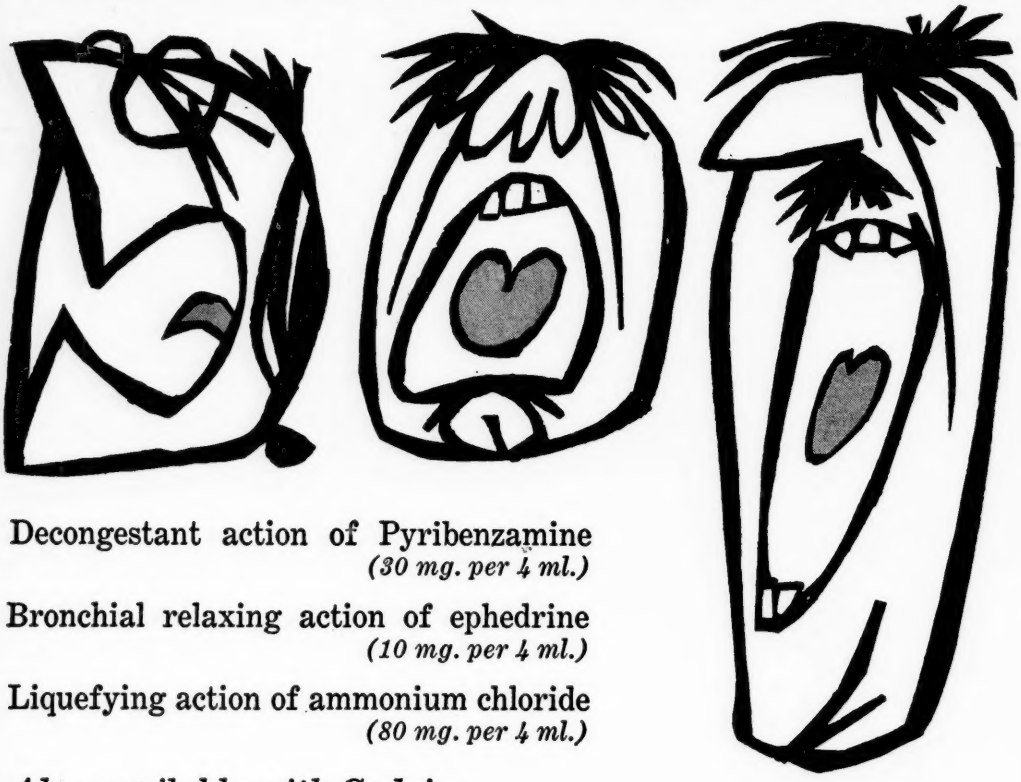

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The Journal of Allergy

VOL. 26 MARCH, 1955 NO. 2

Original Articles

AUTOIMMUNOLOGIC DISEASE

PHILIP A. CAVELTI, M.D., PALO ALTO, CALIF.

THE concept of autoimmunologic disease comprises conditions which are due to the pathogenic effects of antibodies produced by an individual against components of his own tissues. The existence of such conditions in man has been established beyond doubt, particularly in the field of hematology. However, there is also some experimental evidence, to be reviewed here, indicating that a number of important diseases in other fields may be of this type.

EXPERIMENTAL BACKGROUND

Disease-Producing Effects of Antitissue Antibodies of Heterologous Origin.—The effects in vivo of serum prepared against practically every organ and tissue of the body have been studied in the past. Definite results have been obtained with antiserum against bone marrow,[58] leukocytes,[12] and platelets[2, 51] produced by immunization of animals of a foreign species with these materials. Upon injection in homologous recipients, such serums caused leukopenia, thrombocytopenia, and hemorrhagic purpura-like lesions. Likewise, hepatic lesions have been produced with antiliver serum.[23, 55] The most extensive investigations along this line, however, have been carried out with antikidney serum. So-called nephrotoxic nephritis (Masugi nephritis) is easily obtained by injection of an antikidney serum prepared by immunization of animals of a foreign species with kidney substance of the recipient species.[20, 26, 55, 56, 72, 79, 80, 81, 86] The pathologic and clinical features of this renal disease have been thought to resemble human glomerulonephritis rather closely,[20, 56] particularly when the lesions are produced in rabbits. When rabbit anti-rat-kidney serum is given to rats or when other mammals receive antikidney serum produced in a foreign mammalian species, the renal lesions have been found to develop immediately, that is, from

Presented before the Section on Allergy at the eighty-third Annual Session of the California Medical Association, Los Angeles, May 9 through 13, 1954.

Received for publication July 7, 1954.

within hours to a day or so.[35, 79, 89] Therefore, these renal changes must be due to the direct effect of the antikidney antibodies. On the other hand, when antikidney serum against mammalian kidney (rabbit, dog) produced in an avian species (duck, chicken) is used, there is usually a time interval of about a week between the serum injection and the onset of the renal disease.[20, 26, 72] The reason for this is not clear, but it seems that an active immunologic response on the part of the recipient animal occurs. Kay[41, 42] has suggested that the localization of antikidney antibody in the kidney does not suffice to produce nephritis, but that the renal lesions develop only after the recipient animal has formed precipitins against the foreign antikidney serum which react with the antikidney antibody already bound in the kidney. Although apparently the avian antikidney serum is, per se, incapable of producing renal lesions, the antikidney antibodies localize in the kidney immediately after injection, regardless of whether avian or mammalian nephrotoxic serum is used.[67] This is evidenced by the prevention of renal disease in one kidney if its arterial blood supply is shut off for fifteen to thirty minutes following intravenous injection of antikidney serum[72, 73] and especially also by Pressman's demonstration of localization in the glomeruli of antikidney antibodies labeled with radioactive iodine.[22, 63-66]

Ehrich and his co-workers[21] have suggested that two different renal lesions may be evoked by antikidney serum, the one type which follows immediately upon the injection of nephrotoxin being characterized mainly by alteration with thickening of the glomerular basement membrane with but little inflammatory or proliferative changes (that is, a nephrotic lesion), while the other type which develops after an interval of one week closely resembles acute human glomerulonephritis. According to Ehrich, the same rabbit anti-rat-kidney serum can produce either type of lesion in rats, a large dose resulting immediately in the nephrotic type and a small dose leading, after an interval, to the nephritic type. Others[52] also have pointed out that there is a considerable variability in the effects of individual antikidney serums, even when produced in the same species. Different duck anti-rat-kidney serums have been found to exert either the immediate or the delayed nephrotoxic effect.[3, 31] Pressman and his associates[67] have called attention to the fact that duck anti-rabbit-kidney antibody, when combining with rabbit kidney, does not fix complement, thus perhaps explaining the usual lack of direct nephrotoxicity of this serum.

The kidney antigen responsible for nephrotoxic nephritis appears to be species-specific and located in the glomerulus.[29] Nephrotoxic sera are produced only by immunization with renal cortex,[34] not with medulla. Absorption of antikidney serum with a suspension of isolated glomeruli removes the nephrotoxic antibody.[82] On the other hand, substances closely related to the glomerulus antigen appear to be present in vascular endothelium, or at least in the vascular bed elsewhere. In Pressman's experiments,[66] labeled antikidney serum having a predilection for the glomeruli also localized to a smaller extent in the vascular bed of liver and lung. Elution and reinjection of the localized antibody resulted in a predilection for the organ of original localization, but again

with some cross reacting with other locations, indicating close immunologic relationship of the vascular bed of various organs but not identity. Nephrotoxic sera which caused nephritis on injection also have been produced by immunization with lung,[13] placental,[78] and aortic tissue.[85]

It is a remarkable and puzzling fact that often a single injection of a moderate, or even a small, amount of nephrotoxic serum is sufficient to lead to a chronic, progressive, renal disease extending over months or even years. This suggests that the noxious agent must persist or newly arise in the recipient's body. Pressman has shown that the labeled antikidney antibodies localized in the kidney have a half life of 20 days,[65, 67a, 67b] thus perhaps being so located as to be inaccessible to phagocytosis. It seems doubtful, however, that this would wholly explain the chronicity of the lesions, because in some animals chronic progressive disease may develop after a latent period of several months following the early acute renal manifestations. Autoantibody formation arising from modification of kidney substance damaged by the nephrotoxin in such a way that it becomes an antigen could conceivably be the cause.

Antigenicity of Homologous Tissue Constituents—Experimental Autoimmunologic Diseases.—While ordinarily tissue constituents are not antigenic in the same animal or within the same species (in the latter case, with the exception of isoantigens), it has been known for some time that modification of such constituents and especially combination with foreign proteins can render them antigenic. In studies on the nature of the Wassermann antibody, Sachs, Klopstock, and Weil[70] found that rabbits injected with alcoholic extracts of normal rabbit organs in mixture with hog serum developed antibodies reacting serologically with plain alcoholic organ extracts. Heiman and Steinfeld[33] obtained antibrain antibodies in rabbits immunized with alcoholic extracts of rabbit brain mixed with hog serum. Schwentker and Rivers[76] produced complement-fixing antibodies to rabbit brain in rabbits injected with vaccinia virus-infected rabbit brain and even with autolyzed sterile rabbit brain, but not with fresh brain material alone. Burky[5] injected rabbits with staphylococcus toxin from a beef lens broth and obtained skin reactions with plain lens material, including rabbit lens. Traumatization of the lenses of these rabbits resulted in ophthalmia. Similarly, rabbits treated with staphylococcus toxin grown on rabbit muscle broth showed skin reactions to plain rabbit muscle extract. Hecht, Sulzberger, and Weil,[32] using rabbit skin in conjunction with staphylococcus toxin obtained in the serum of rabbits, so treated precipitins for rabbit skin extract. Schwentker and Comploier[77] found complement-fixing antibodies against rabbit kidney in rabbits injected with rabbit kidney emulsion plus staphylococcus toxin and, to a slighter extent, also when streptococcus toxin was used in mixture with kidney.

In most of the studies mentioned so far, no organic lesions have been obtained as a result of the antibodies produced. In our experiments[6-8] rabbits were injected with rabbit kidney mixed with killed group A streptococci. High titers of antibodies to rabbit kidney, as demonstrated with the collodion particle agglutination technique, were obtained, and a few rabbits developed acute

glomerulonephritis. In rats similarly immunized with rat kidney plus streptococci, glomerulonephritis was produced with considerable frequency. In most cases, antikidney antibodies could be demonstrated only during a short period before the onset of the disease, suggesting that they disappeared from the blood by reacting with the animal's kidney. In a number of cases the acute nephritis was followed, without further treatment, by chronic nephritis progressing over a period of many months. Subsequent to our reports, Frick[28] obtained complement-fixing antikidney antibodies and glomerulonephritis in rats immunized with rat kidney plus killed tubercle bacilli and Freund's adjuvant. Donoso, Rodriguez, and Steiner[18, 19] found that rats treated with perfused kidney material from rats injected repeatedly with killed streptococci formed complement-fixing antikidney antibodies and the serum of these rats, when injected to normal rats, produced glomerulonephritis. Sprunt, Rogers, and Dulaney[83, 84] claimed similar results in analogous experiments with staphylococcus toxin in rabbits. In analogy with our experiments with kidney tissue,[9, 10] we immunized rats with mixtures of killed streptococci and rat heart or connective tissue material and obtained antibodies reacting in vitro with extracts of rat heart and connective tissue. Histologically endocardial and myocardial lesions of the rheumatic type were found. Cardiac lesions obtained in rats, rabbits, and guinea pigs immunized with homologous heart, with or without streptococci, subsequently also were reported by Jaffé and Holz.[36] Frick[28a] produced antibodies reacting by complement fixation with rat heart muscle in rats injected with rat heart plus killed tubercle bacilli in Freund's adjuvant. In many of these animals he found myocardial lesions. Using rat lung tissue instead of heart, he obtained anti-lung antibodies and pneumonic lesions in rats.[28b] Glynn and Holborow[28c] made chondroitin sulfuric acid antigenic by combination with group A streptococci and observed arthritic lesions in rabbits immunized with the complex.

Extensive investigations have been carried out in recent years on the production of lesions of the central nervous system by immunization with brain or spinal cord material. These were initiated by Rivers and his associates[68, 69] in 1933 by the production of demyelinizing inflammatory lesions in monkeys immunized with rabbit brain suspension or alcoholic extracts of rabbit brain over prolonged periods of time. The subsequent use of brain or spinal cord material in mixture with Freund's adjuvant and killed tubercle bacilli or other acid-fast organisms greatly accelerated and intensified these lesions, so that now they can be produced by a few injections or a single injection of the material in a high percentage of animals.[1, 27, 37-40, 44, 45, 53, 54, 57, 59, 88] The disease has been produced in various animal species such as monkeys, rabbits, rats, guinea pigs, and mice. The lesions appear about five days to several weeks after the injection and are characterized by perivascular inflammatory changes and demyelinization, and clinically by paralysis. They have been considered to resemble those of acute phases of multiple sclerosis in man and of postvaccinal and certain types of postinfectious demyelinizing encephalomyelitis. The responsible antigen is a component of myelin[1] and is highly organ-specific but not

species-specific, since heterologous and homologous, and even autogenous, brain materials are equally effective. It seems likely that the disease is due to the effect of antibrain antibodies formed by the animal in respone to the immunization. Such antibrain antibodies have been demonstrated in the blood of the animals, although there was no consistent correlation between the titer of the antibodies and the extent of the lesions.[45] Delayed skin reactions to brain material without adjuvants in the immunized animals also have been reported, sometimes accompanied by reactivation of the central nervous system manifestations.[88] It has been noted that animals injected with homologous brain material with adjuvants developed encephalomyelitis in a high incidence but failed to show antibrain antibodies in the serum, while animals given heterologous brain substance with adjuvants showed antibrain antibodies, even though the incidence of encephalomyelitis was lower.[45] This would suggest that the true autoantibodies react with the animal's brain and disappear from the blood, while antibodies formed against species-specific heterologous brain constituents are not absorbed and, therefore are demonstrable in the serum. This also would explain failure of production of encephalomyelitis by passive transfer to normal animals of serum from animals treated with brain plus adjuvants.[39]

HUMAN AUTOIMMUNOLOGIC DISEASE

Diseases of the Blood.—It is beyond the scope of this discussion to review the complex field of autoimmune hematologic diseases, but the fact of the common occurrence of such conditions in man strongly implies that autoimmunologic mechanisms may be equally important with regard to cells or intercellular substance of other tissues and organs. The first recognition of an autoimmunologic human disease was made in 1904 by Donath and Landsteiner,[17] when they showed that paroxysmal hemoglobinuria is due to a hemolysin which reacts with the patient's red blood cells in the cold and lyses the cells in the presence of complement at body temperature. In recent years it has been definitely established that many cases of hemolytic anemia are due to autoantibodies against the patient's red blood cells, that autoantibodies against leukocytes cause granulocytopenia and that idiopathic thrombocytopenic purpura results from platelet autoantibodies.[15, 16, 24, 25, 90] Some of the serologic dynamics of these conditions are highly revealing and may have important implications with regard to autoimmunologic disease mechanisms in general. The cellular antigens involved appear to be species-specific and probably cell-specific but distinct from the blood group isoantigens. In the majority of cases erythrocyte autoantibodies, for instance, react with all human erythrocytes. In some instances, however, a reaction restricted to the patient's own cells has been observed.

The autoantibodies present in the blood may be free in the serum or they may be attached to the respective cells, as shown by the Coombs antiglobulin test. Usually both are present, but when only one is present it is, in most cases, the one that is attached to the cells.[16] This would seem to indicate that as the antibody is produced and appears in the blood it reacts with the cells attaching itself to them, but that after all cells have been saturated it accumulates in the serum as free antibody. Thus, the free antibody would constitute an overflow.

In some cases a large proportion of the total serum gamma-globulin fraction is made up of autoantibody. Sera containing large amounts of platelet autoantibody, when injected to normal persons, have caused severe hemorrhagic crises with acute thrombocytopenia.[30]

As to the pathogenic effect of these autoantibodies, it appears that the autoantibody-antigen union in vivo is only the first step in the sequence leading to destruction of the respective blood cell. Splenectomy, which often brings on a definite remission or even a cure, does not always abolish the serologic findings, the autoantibodies remaining demonstrable. Thus, it would seem that the autoantibody prepares the cells for destruction by other agents or biochemical mechanisms, perhaps comparable to the effect of opsonins in phagocytosis of bacteria, and that by splenectomy one of the major organs concerned with the destructive processes is removed. Treatment with ACTH and cortisone, on the other hand, has been reported to be followed by serologic changes[16] and therefore appears to have some inhibitory effect on antibody production and possibly on the reaction between antibody and antigen.

GLOMERULONEPHRITIS

Autoantibodies reacting in vitro with extracts of human kidney have been found in the serum of patients with glomerulonephritis by several independent workers. Lange and associates,[47, 48] using the collodion particle technique, obtained positive results in 58 per cent of the cases of acute glomerulonephritis and in 100 per cent of those of chronic glomerulonephritis. Pfeiffer and Bruch,[60-62] by means of the same method, found antikidney antibodies to be present in the blood of 72 per cent of their patients with acute glomerulonephritis and 83 per cent of their patients with chronic glomerulonephritis. Both groups of workers found considerably higher titers in chronic than in acute nephritis. The latter investigators also obtained positive results in relatively low titer in malignant nephrosclerosis, while cases of essential hypertension were negative. Because of the lower titers in acute, as compared to chronic, glomerulonephritis, Pfeiffer and Bruch favor the view that the antikidney antibodies are not the cause of acute nephritis but are, rather, the result of an immunologic response to breakdown of kidney tissue by the disease and possibly the cause of protracted chronic renal disease. However, in view of the rapid fixation in the kidney of antikidney antibodies injected into animals, it would be expected that in the early stage of human acute nephritis, likewise, most of the antibody is bound to the kidney, and that it accumulates in the serum only gradually as the kidneys become saturated with antibody. As mentioned previously, the same phenomenon is observed in autoimmune hematologic diseases. Furthermore, Lange and his co-workers,[49] in confirmation of previous reports, have shown that there is a pronounced drop of complement in the blood in acute nephritis, indicating the occurrence of a massive antigen-antibody reaction—presumably of antikidney antibody with kidney substance. Autoantibodies to kidney in acute and chronic glomerulonephritis also have been demonstrated by Vorlaender,[87] using complement fixation.

RHEUMATIC FEVER

There are reports in the literature of serologic reactions between serum of rheumatic patients and human tissue materials. Brockmann and associates[4] obtained complement fixation reactions using extracts of heart and liver from patients dead of rheumatic fever. Schlesinger[75] and others described agglutination by rheumatic serum of particles found in pleural and pericardial exudates of rheumatic patients. At the time, these results were considered in the light of a virus etiology of rheumatic fever. In our own studies[11] agglutination of collodion particles sensitized with an extract of normal human heart by serum of acute rheumatic patients was observed, but these results could not be reproduced consistently with extracts of various heart specimens. Later[11a] a quantitative type of complement fixation test on rheumatic serums gave positive results with extracts of various normal and rheumatic human heart and liver samples in a small number of cases. Controls, including sera from poststreptococcal states, were negative. With serum of patients with rheumatoid arthritis Lansbury and co-workers[50] observed precipitin reactions and complement fixation by using as antigens extracts of normal or rheumatoid synovial tissue or lymph nodes. However, these authors considered it doubtful that the reactions were immunologic in nature. Vorlaender[87a] found complement-fixing autoantibodies to heart and other tissues in the serum in rheumatic fever and in rheumatoid arthritis. In acute rheumatic recurrences, the serologic tests usually became positive somewhat after the onset of the clinical manifestations. This led the author to conclude that these antibodies were a result, not the cause, of the rheumatic tissue damage. While this may be so, one would also expect that pathogenic autoantibodies would react first with the tissues and that only later an overflow would become serologically demonstrable, especially if the serologic method is relatively insensitive. Vorlaender[87, 87a] also described autoantibodies to liver in infectious hepatitis, and similar findings were mentioned by Scheiffarth[74] in infectious hepatitis and hepatic cirrhosis.

It seems likely that antibodies reacting serologically with homologous tissue constituents also can arise as a response to tissue damage by a disease. The serologic findings in chronic nephritis, for instance, may well reflect a coexistence of such antibodies with true, primarily pathogenic autoantibodies, while, in certain other conditions, only the former may be present. To distinguish the two types of autoantibodies would seem to be very difficult until the respective antigens can be more clearly defined.[74a]

CENTRAL NERVOUS SYSTEM DISEASES

There is, as yet, no direct evidence of antibrain antibodies in multiple sclerosis or related conditions, except for old reports by Sachs and Steiner,[71] who described complement-fixing antibodies in the serum in acute phases of multiple sclerosis with brain material from multiple sclerosis patients, as well as arteriosclerotic and other brains. The encephalitis following rabies vaccination with virus grown in rabbit brain appears to be a direct parallel to experimental allergic encephalomyelitis and, in this condition, antibrain antibodies have been demonstrated in the patient's blood.[43, 46]

DISCUSSION

One of the most important questions regarding autoimmunologic disease mechanisms concerns the nature of the inciting stimulus leading to antigenicity of autogenous tissue substances and thus to autoantibody production. Clinically, in acute glomerulonephritis the connection with a preceding streptococcal infection is well established and the experimental findings cited would suggest that the streptococcus or its toxins may act upon renal substance, particularly of the glomeruli, in such a way as to render it antigenic. In view of the apparent close relationship of the glomerulus antigen with substances of vascular structures in general, such action of streptococcal products on vascular tissues anywhere in the body might suffice. A similar role could be attributed to streptococci in rheumatic fever, where an effect inducing antigenicity to components of connective tissue would be suggested. In postrabies vaccination encephalitis, it seems obvious that antigenicity on the part of a central nervous system constituent of the vaccine arises from the action of the virus.

On the basis of experimental immunologic facts, such antigenicity could occur when foreign protein from microorganisms, toxins, etc., combines with a tissue hapten after breakdown of tissue protein-hapten complex. This view is supported by the fact that alcoholic extracts of brain substance can be used in conjunction with a foreign protein to produce antibrain antibodies and encephalomyelitis; it is supported also by Cole's findings that nephrotoxin can be neutralized by a renal hapten obtained by trypsin digestion of kidney material.[14] Another mechanism might be the modification of a tissue substance (protein) itself in such a way that it becomes sufficiently foreign to act as an antigen and yet retains enough immunologic resemblance to the original material that cross reaction occurs on the part of the antibody formed. Such a change might be produced by various chemicals, drugs, and perhaps also by physical influences (heat, cold, radiation, etc.). Autoimmunologic diseases of the blood have been observed, in some cases, to arise following the use of certain drugs (Sulfonamides, Sedormid, etc.), although in most cases of this group there seems to be no clue as to the agent inciting the autoantibody formation. The latter is also true of multiple sclerosis.

It would seem reasonable to expect that autoimmunologic disease-producing mechanisms constitute a general immune-pathologic principle and thus exist with regard to many cells, organs, or systems, their type and the localization of the lesions depending on the specificity of the particular antigen-antibody system involved. Aside from rheumatic fever, the inclusion in this group of other collagen diseases such as certain cases of periarteritis nodosa, lupus erythematosus, rheumatoid arthritis, and certain diseases of other organ systems might be speculated upon, but as yet without any experimental basis. Serologic and other immunologic studies in diseases of this type, using tissue antigens, are urgently needed. However, as is evident from studies in autoimmunologic hematologic conditions, serologic tests with the blood are not necessarily conclusive. It might be necessary to search for the antibody in the tissues where it is bound, as in the demonstration of antibody bound to red cells by the Coombs antiglobulin test.

With regard to therapeutic possibilities, autoimmunologic disease would seem to present a most difficult problem. Where infection is the inciting cause, its prophylaxis or its eradication in an early stage is effective, as in prevention of recurrences of rheumatic fever. Blocking or inhibition of antibody production or of the antigen-antibody reaction or a protection of the cells from the damaging effects of antigen-antibody reactions are other possibilities. Perhaps adrenocortical therapy acts by some of these mechanisms. The most desirable approach, however, would seem to be specific immunologic treatment. When more is known about the specific mechanisms and the nature of the tissue antigens involved, it would seem, at least theoretically, possible to neutralize autoantibodies as they are formed by administration of the appropriate tissue antigen, particularly if the latter could be obtained as a true hapten, not capable of inciting antibody formation. Investigation of the possibility that administration of tissue antigens in appropriate dosage, analogous to the present-day specific treatment of ordinary allergy, might serve to desensitize or to incite formation of a blocking antibody also would seem worth while.

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ALLERGENS IN VEGETABLE OILS

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THE parenteral administration of vegetable oils in man may provoke a sensitization easily demonstrated by intracutaneous testing.[6] These oils contain allergens which certainly are not specific for each kind of oil, since intracutaneous tests with different oils have been shown to yield cross reactions.[6] The skin tests are followed by the same kind of local reactions when the oils are entirely refined, that is, completely freed of proteins, carbohydrates, and free fatty acids. Therefore, such impurities can be discarded as possible causes of sensitization by natural vegetable oils. It must be admitted, therefore, that the allergen is either a constituent of the fatty oil by itself, or a lipoid with very similar physical—chemical properties which was not separated from the glycerides during the refining procedure. The purpose of this investigation is to test the hypothesis that triglycerides may be the allergens contained in vegetable oils. This can be done by examining the allergenic properties of chemically pure, synthetic glycerol-fatty acid esters.

MATERIAL AND METHODS

Synthesis of Chemically Pure Triglycerides.—Synthesis of glyceryl trioleate (triolein) and glyceryl tripalmitate (tripalmitin) were carried out by the procedure of Belluci,[2] while glyceryl tributyrate (tributyrin) was obtained by the usual esterification technique.

The absolute glycerol used for all esterifications was purified by twofold vacuum distillation.

Chemically pure oleic acid was obtained by twofold vacuum distillation and further refining the oleic acid by lead and lithium precipitation, according to the method of Täufel and Seuss.[13] The lithium salt of the oleic acid was recrystallized as many times as necessary to obtain a perfectly white salt and an absolutely colorless concentrated solution. The oleic acid liberated from its lithium salt was found to have the expected acid and iodine values.

Chemically pure palmitic acid was obtained by several recrystallizations of pure commercial palmitic acid, until the substance yielded a stable and well-defined melting point. Analysis of the synthetically obtained glyceryl trioleate and tripalmitate yielded values in agreement with the theoretical saponification and iodine values. The melting point of the tripalmitin was 64° C.

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This study was supported by a grant from the Swiss National Foundation for Scientific Research.

Received for publication Sept. 15, 1954.

For the synthesis of glyceryl tributyrate, pure butyric acid anhydride was used. The tributyrin then obtained was further purified by vacuum distillation.

Sensitization Technique.—Sensitization experiments were performed with chemically pure butyric acid, tributyrin, tripalmitin, and triolein, obtained as described in previous paragraphs of this article. The liquid preparations were given intracutaneously to healthy volunteers in single doses of 0.1 c.c. every twenty-four hours except on Sundays. Solid tripalmitin was rubbed into scarified skin areas. This technique was adopted to avoid dissolution of tripalmitin in fat solvents, which might in themselves be biologically active. The sites of injection or scarification were controlled daily with regard to redness and swelling. Total leukocyte and eosinophil counts with the phloxine method were done every second day.

RESULTS

1. *Butyric Acid.*—The intracutaneous injection of butyric acid provoked a strong local irritation. During injection a burning pain was felt, which lasted for several minutes. Then an erythematous papule about 10 mm. in diameter appeared and lasted for about twenty-four hours. During a series of twelve injections in fourteen days, this immediate reaction occurred after each injection, without any change in timing or local aspect. The total leukocyte and eosinophil counts remained unchanged. The experiment was stopped after two weeks because of painful local reaction.

2. *Tributyrin.*—In this experiment a painful local reaction was also elicited, along with an erythematous papule, 3 to 4 mm. in diameter, which disappeared within twenty-four hours. During an experiment of three weeks' duration, the same cutaneous reaction occurred each time. The total leukocyte and eosinophil counts remained unchanged. It is probable that the local reaction was due to the small quantities of free tributyric acid contained in the injected material, as tributyrin very rapidly undergoes a slight hydrolysis during storage.

3. *Tripalmitin.*—This ester, applied by the scarification method, did not produce any local reaction or any change of the total leukocyte and eosinophil counts during an experiment lasting five weeks.

4. *Triolein.*—When injections were performed with triolein, stored for some time and containing, therefore, small quantities of free oleic acid, a burning pain was felt and a papule, 5 to 6 mm. in diameter, appeared within one hour and disappeared after twenty-four hours. When freshly prepared triolein was injected, however, no pain was evoked and no trace of local reaction could be observed (Fig. 1) ten minutes, thirty minutes, one hour, two, twelve, twenty-four, forty-eight and seventy-two hours after its first application. Twenty-five further injections could be given without any reaction. However, on the twenty-eighth day of the uninterrupted series of skin tests, a local redness and swelling, 8 mm. in diameter was observed within twenty-

four hours after injection (Fig. 2). After that, each skin test was followed by an increasingly strong local reaction so that during the fifth week of the injection series, papules 12 to 15 mm. in diameter were provoked. At that time, analogous reactions were observed at the sites of the early injections, which previously had not been followed by any local signs (Fig. 3). Redness and swelling remained unchanged for several days and disappeared only

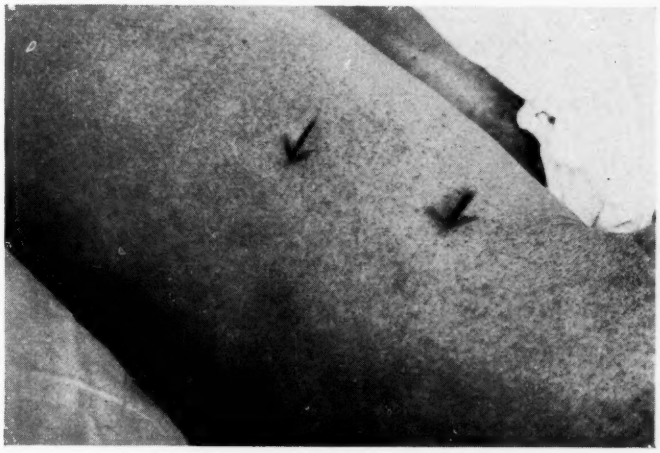

Fig. 1.—Sites of initial intracutaneous applications of 0.1 c.c. triolein, twenty-four and forty-eight hours after injection. No reaction is seen.

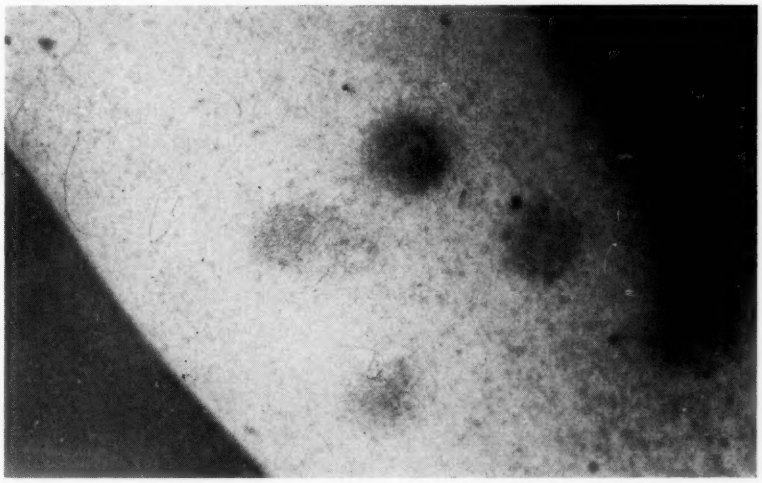

Fig. 2.—Sites of four serial intracutaneous triolein injections (twenty-fourth to twenty-eighth injections) in the course of the fifth week of the series of injections. Redness and swelling at the sites of injection (left upper arm, same person as in Fig. 1).

in the course of three weeks. A total of thirty-one injections of 0.1 c.c. was administered in the course of this experiment. The total leukocyte and eosinophil counts did not change significantly. The highest eosinophil count was 185 cells per cubic millimeter (4 per cent).

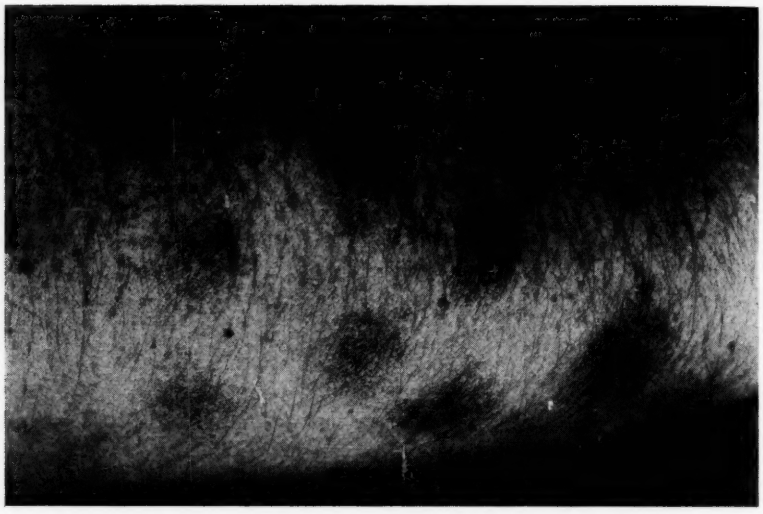

Fig. 3.—Late flaring up at the sites of previous injections. The wheals occur at the sites of intracutaneous injections given three weeks earlier (right forearm, same person as in Figs. 1 and 2).

DISCUSSION

It has been shown that repeated injections of triolein may produce sensitization. This sensitization can be followed up by observing the behavior of cutaneous oil papules. The intracutaneous oil deposits do not elicit any local reaction until, after several weeks of continuous skin testing with the oil, the injections produce a cutaneous reaction of the tuberculin type, that is, redness and swelling occurring within twenty-four to twenty-eight hours after injection. Simultaneously with the first positive skin tests, similar reactions occur at the sites of earlier injections, which originally had not been followed by any reaction. This phenomenon is due to the slow absorption of the triolein, which remains for several weeks in the skin. This slow absorption also explains why the cutaneous signs do not disappear after a few days as with tuberculin, but last for several weeks. Allergic skin reactions following testing with refined vegetable oils behave identically.[6] This sensitization by refined vegetable oils and by chemically pure synthetic triolein shows that allergy to plant oils is due to the fatty acid glycerides and not to chemical impurities.

Triolein probably behaves as a hapten, as it is not likely that such a simple substance has the properties of a complete antigen. It might be significant that it is the triglyceride of an unsaturated acid, the oleic acid, that is the sensitizing agent and not the glyceride of the saturated palmitic and butyric acids. This agrees with the observations made by Hass[9] that unsaturated oil fractions are biologically more active than saturated fractions.

The behavior of the blood eosinophils after application of vegetable oils and chemically pure synthetic fatty acid glyceride is of interest. Protracted intramuscular injections of sesame and almond oils in man and guinea pigs provoke a significant rise of the number of blood eosinophils.[3, 5-8, 10] During

daily administration of 1.0 to 2.0 c.c. oil to man and 0.25 c.c. to guinea pigs, the eosinophil count begins to rise after about ten days, reaches values of 20 to 80 per cent, and drops again to normal within three to four weeks after cessation of the intramuscular oil injections. This blood eosinophilia is accompanied by a high-grade bone marrow eosinophilia. After the return of the eosinophil count to normal, a single oil injection does not produce any rise of the eosinophils. However, a new protracted series of oil injections produces again an eosinophilia analogous to the first one. This blood eosinophilia has the characteristic features of the resorption eosinophilia, as defined by Essellier and co-workers,[3, 4, 6, 8] that is, constant period of latency, progressive rise and drop, and proportionality of the maximum number of blood eosinophils to the amount of oil injected, that is, the intensity of the eosinopoietic stimulus. These differential features leave no doubt that the eosinophilia occurring after intramuscular oil injections is not of an allergic nature.

In contrast with the protracted intramuscular application of relatively large amounts of vegetable oils, the intracutaneous sensitization with almond and sesame oil[5, 6] and with triolein is not accompanied by any significant rise of the blood eosinophil count. This behavior of the blood eosinophils is well accounted for by the difference in the quantities of oil injected. In the course of the series of intramuscular injections leading to a resorption eosinophilia, total amount of 12 to 173 c.c. of oil were injected. In the experiments with intracutaneous triolein, however, the total amount of oil given was only 3.1 c.c. It is obvious that the resorptive and inflammatory reactions elicited by such small quantities of oil are not sufficient to produce a significant rise of the blood eosinophils. The different behavior of the blood eosinophils during intracutaneous and intramuscular administration of oil is therefore easy to understand. The fact that the positive skin tests are not accompanied by a blood eosinophilia does not speak against the allergic nature of these reactions. Although the eosinophil granulocytes contain serologically specific substances,[12] it is well established that eosinophilia is not an obligatory symptom of the allergic reaction.[1]

SUMMARY

Serial intracutaneous injections of chemically pure, synthetic glyceryl trioleate (triolein) lead to the same allergic reactions as vegetable oils. The sensitization by natural vegetable oils can be attributed to fatty acid triglycerides and not to impurities. This sensitization is not accompanied by a rise of the blood eosinophils. The absence of eosinophilia fits in with the known fact that eosinophilia is far from constant in allergy and with our finding that large amounts of oil are needed to produce a resorption eosinophilia.

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ESTIMATION OF DAILY CHANGES IN THE SEVERITY OF BRONCHIAL ASTHMA

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AS PART of a larger study on asthma,* attempts have been made to improve methods of assessing the severity of the disease as it waxes and wanes over a period of time. Severity may be measured or estimated in a number of ways: (1) by the intensity and number of musical râles and rhonchi heard in the chest with the stethoscope; (2) by tests of pulmonary function; (3) by the amount of medication the patient requires; and (4) by the intensity of symptoms as reported by the patient.

Examination of the chest gives some indication of severity, but we have not found this to be reliable, even though the presence of asthma may be detected so very readily by this means. The auscultatory signs of asthma, which are difficult to express quantitatively anyway, are increased when there is increased ventilation for any reason and they are likewise decreased when the minute volume falls. Furthermore, in the course of giving various drugs for the relief of asthma, we have observed on occasion with the patient at rest, an intensification of the auscultatory signs, even though there was subjective relief and evidence of improvement as judged by an increase in the vital capacity. The administration of bronchodilator drugs (isopropylarterenol, aminophylline) may bring about an increase in ventilation,[1] but it is not clear whether or not this explains the intensification of physical signs just referred to.

Tests of pulmonary function not only reveal abnormalities, but also yield data which may be expressed quantitatively in numerical terms. To be sure, many of these tests are cumbersome and cannot be done at frequent intervals. Among the simpler procedures, the timed vital capacity appears to be satisfactory[2, 3] and demands little of the patient in the way of time, effort, and cooperation. Even this can be done only once or twice weekly in most patients and, as the intensity of asthma tends to fluctuate from day to day, such measurements may not be representative of the intervening periods.

If a patient takes medication only for the relief or control of symptoms as they arise, the amount of medication taken will provide an index of the intensity and frequency of asthmatic manifestations. However, the amount of medication taken may be influenced by factors other than the intensity of symptoms.

Estimation of severity based on the patient's answers to questions asked at intervals of days or weeks has been unsatisfactory for our purpose, depend-

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Presented in part at the Tenth Annual Meeting of The American Academy of Allergy, Houston, Texas, Feb. 1 to 3, 1954.

Received for publication July 19, 1954.

*Supported by a grant from the U.S. Public Health Service.

ing as it does on the patient's memory, the physician's ability to weigh correctly the descriptive terms used by the patient, and, no less important, his ability to record these accurately. In this respect our records, upon later review, have given us inadequate information for our purposes, a deficiency which we reluctantly admit.

Name: Month: Year:

	Medication	(Day of Month)
1	Nebulizer	
	Ephedrine	
	Epinephrine s.c.	
	Aminophyllin p.r.	
	Aminophyllin i.v.	
	ACTH	
	Cortisone	
	Hospitalization	
2	"Tightness"*	
	Choking*	
	Wheezing*	
	Cough*	
	"Raising"*	
3	"Out of breath" stairs*	
	"Out of breath" walk*	
	"Out of breath" rest*	
4	Work, days missed	
	Daily activities, %	
	Appetite, %	
	Sleep, %	
See text for method of scoring	1	
	2	
	3	
	4	
	Total	

*Once in 24 hrs. =✓; off and on =✓✓; continuous =✓✓✓

Fig. 1.—A sample score sheet with medication indicated as required by Patient 3.

In short, dependence on any one of these criteria for estimation of the severity of asthma is clearly open to error. In this study we have attempted to gain a better quantitative estimate by combining several approaches. Accordingly, the subjects were selected from a group of asthmatic patients undergoing intensive study.

We have done simple tests of pulmonary function at approximately weekly intervals and, in addition, we have obtained from the patient a daily record of the amount of medication taken and the number and degree of asthmatic symptoms.

MATERIALS AND METHODS

The tests of pulmonary function consisted of measurement of the vital capacity before and after the administration of a bronchodilator drug (isopropylarterenol 1:200 by aerosol) made with a kymograph moving at 0.56 cm. per second so as to obtain a record of the expiratory rate during measurement of the vital capacity.[3] The volume expired in the first second was selected as an index of the expiratory rate and this and the vital capacity were converted to a percentage of the expected normal, corrected for height, sex, and age.[4]

A score sheet, of which Fig. 1 is an example, was given to each patient to be filled out by him daily and by which he could describe with numbers and checks: (1) the amount of medication taken; (2) manifestations and symptoms of asthma; (3) the occurrence of dyspnea at varying levels of activity; and (4) the patient's state of well-being as judged by his ability to work, daily activities, appetite, and sleep.

The various medications taken by this group of patients were scored by arbitrarily assigning to each at the beginning of the study a numerical value proportional to the capacity of each drug to relieve asthma as judged by the three authors (Table I). One use of the nebulizer (epinephrine or isopropylarterenol) was given a score of 1 regardless of the number of inhalations. One capsule or tablet of any ephedrine-containing preparation was assigned a score of 4, epinephrine subcutaneously (regardless of quantity) and aminophylline by rectum, a score of 8; and aminophylline intravenously, a score of 16. The total for a twenty-four-hour period represented that day's score for medication. The administration of cortisone and periods of hospitalization were noted but were not included for scoring purposes.

TABLE I

1. MEDICATION

DRUG	SCORE
Antihistamine	1
Nebulizer	1
Ephedrine	4
Epinephrine, s.c.	8
Aminophylline, p.r.	8
Aminophylline, i.v.	16

2. MANIFESTATIONS

SYMPTOM	DEGREE AND SCORE		
	√	√ √	√ √ √
Wheezing	2	4	8
Cough	1	2	4
"Tightness"	1	2	4
"Choking"	1	2	4
"Raising"	1	2	2

3. DYSPNEA

WHEN OCCURRING	DEGREE AND SCORE		
	√	√ √	√ √ √
Climbing stairs	1	2	4
Walking	2	4	8
At rest	4	8	16

4. GENERAL ACTIVITY AND STATE OF WELL-BEING

	>90%	50%-90%	<50%
Daily activity	0	4	8
Appetite	0	4	8
Sleep	0	4	8
Work or school	days missed		8

√= once in twenty-four hours; √ √ = off and on; √ √ √ = continuous.

The frequency of the various symptoms, of which wheezing is an example, was indicated for each day with one (once in twenty-four hours), two (off and on), or three (continuously) checks (Table I). The intensity of asthmatic symptoms was also judged by the extent to which they interfered with work

or school, with daily activities, and with such physiologic needs as sleep and appetite. The last three were recorded by the patient as a percentage of the amount expected if asthma were absent. The patient was only required to fill in the spaces in the manner described and the scoring was done later by a physician. Patients stated that one to one and one-half minutes were required to fill out the sheet each day, and they were urged to do this at bedtime. The

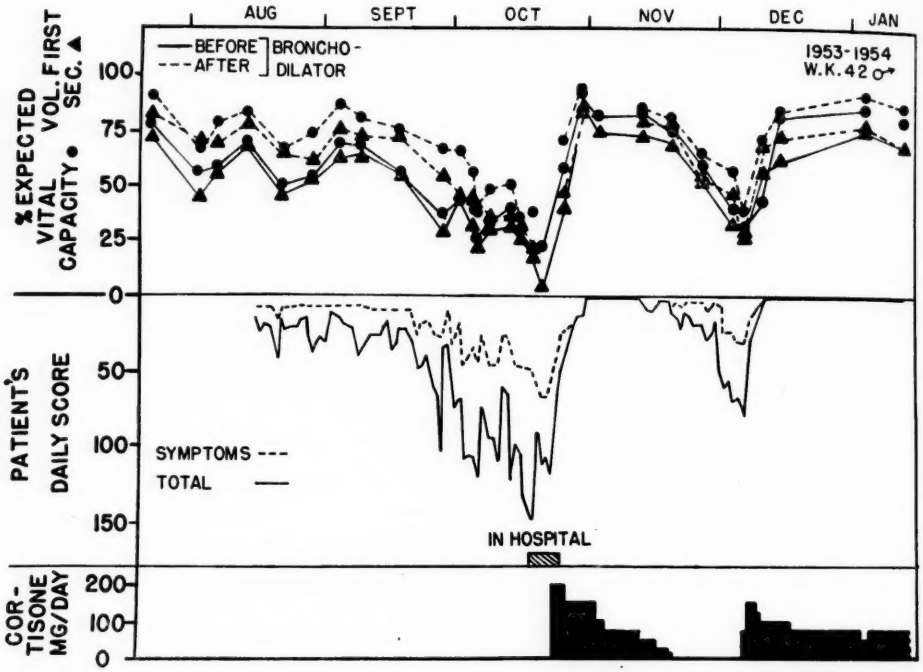

Fig. 2.—Respiratory studies and daily scores for Patient 1.

The upper portion of the figure gives the observed values expressed as the percentage of that expected for a normal subject of the same age and sex,[4] for the vital capacity (circles), and the volume expired within the first second of expiration (triangles), before (solid line) and after (broken line) the administration of six inhalations of a 0.5 per cent solution of isopropylarterenol from a DeVilbiss No. 40 nebulizer supplied with oxygen flowing at approximately 6 liters per minute.

The lower portion of the figure gives the total score (solid line) for each day and the score for the intensity and frequency of asthmatic manifestations (broken line) derived from data recorded by the patient each day (see Fig. 1 and text). The difference between the total score and the score for asthmatic manifestions gives the score for medication.

medication taken and the asthmatic symptoms and manifestations for which amount, degree, or frequency had thus been indicated by the patient, were then scored as shown in Table I. The largest single scores in each of Groups II, III, and IV (Fig. 1) were added, together with the total score for medication, and the grand total represented the score for a twenty-four-hour period.

The patient's own estimation of his disease, as indicated by the manner in which he filled out the score sheet, was compared with measurements of the vital capacity and the volume expired in the first second. The only part played by the physician was explaining the manner in which the sheets were to be filled out and obtaining the spirometric measurements.

A brief history of each of the five patients follows:

CASE 1.—W. K., a 42-year-old, married, white man (Fig. 2), has had asthma for ten years, which was at first intermittent but which has become constant for the past five years. He also has had hay fever from August to September, beginning in 1940. In addition, he has had mild hypertension for the past three to four years. Skin tests have shown reactions to extracts of ragweed and plantain pollen and house dust. The patient has been treated with ragweed pollen and house dust extract for the past year, and he has also received two courses of cortisone. Tests of pulmonary function carried out when the patient was entirely free of symptoms indicated that there was little or no irreversible component to this patient's pulmonary disease. Weight, 213 pounds; height, 5 feet, ten inches.

CASE 2.—J. E., a 42-year-old, white, single man (Fig. 3), had eczema as a baby and developed asthma at 7 years of age. This had been worse from August to November each year, and in August he also has had rhinitis and itching of the eyes, lasting until frost. A sister has asthma.

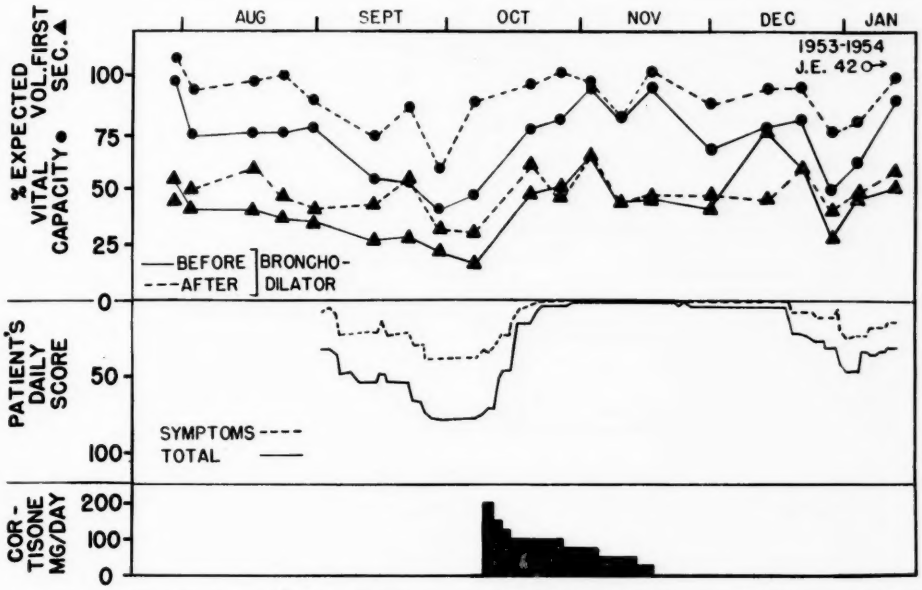

Fig. 3.—Respiratory studies and daily scores for Patient 2. (For explanation, see Fig. 2.)

Skin tests have shown reactions to a number of air-borne allergens, including extracts of grass, ragweed, birch pollen, and house dust. In the past few years asthma has tended to occur during the winter and spring months and has been sufficiently severe on some occasions to require hospitalization. The patient has been treated with injections of ragweed pollen and house dust extract since 1947, and in the past three years he has been treated intermittently with cortisone with excellent relief of symptoms.

A number of inhalation tests with house dust extract have resulted in marked reduction of vital capacity and the induction of asthmatic symptoms. Tests of pulmonary function carried out when the patient was free of symptoms indicated that there was a considerable irreversible component to this patient's pulmonary disease. Weight, 177 pounds; height, 5 feet, 9 inches.

CASE 3.—E. S., a 20-year-old, single, white woman (Fig. 4), developed asthma in 1948 at the age of 15 years. The first attack occurred at her mother's funeral, her mother having

died of asthma. Asthma was intermittent at first, but became continuous in 1950, and the patient has required hospitalization on a number of occasions. Skin tests showed reactions to extracts of ragweed, plantain and grass pollens, molds, and house dust, and the patient has been treated with injections of these, beginning in March, 1953. She received cortisone almost continuously in the year of 1953. While receiving cortisone and at a time when she was free of symptoms, tests of pulmonary function fell within normal limits. Weight, 127 pounds; height, 5 feet, 5½ inches.

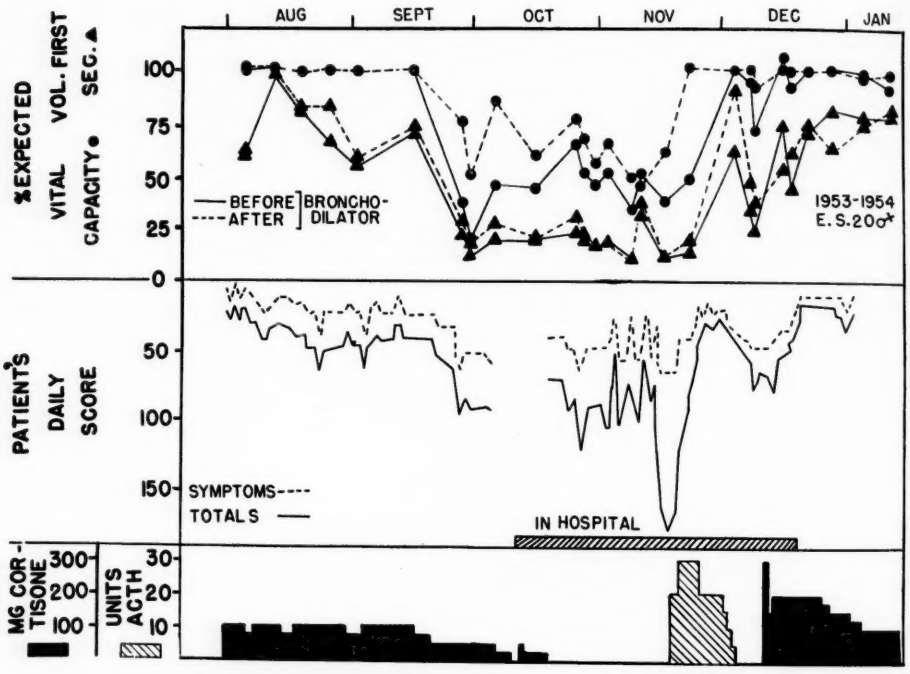

Fig. 4.—Respiratory studies and daily scores for Patient 3. (For explanation, see Fig. 2.)

CASE 4.—E. W. is a 26-year-old Negro housewife (Fig. 5) who has had asthma since infancy, becoming less intermittent as she grew older. As a child she was hospitalized in a tuberculosis sanatarium for two and one-half years, but a diagnosis of tuberculosis was not established. In the past few years asthma has been severe, requiring injections of aminophylline and hospitalization from time to time. Her son also has asthma. X-ray examination of the chest on numerous occasions has shown emphysema and fibrosis but has failed to reveal evidence of tuberculosis. Skin tests have shown reactions to numerous air-borne allergens, including extracts of grass, ragweed and plantain pollen, and house dust, and she is currently under treatment with injections of these. Tests of pulmonary function indicated that this patient has a restrictive and an obstructive defect which appears to be irreversible. Weight, 109 pounds; 5 feet, 2½ inches.

CASE 5.—A. G., a 25-year-old white housewife (Fig. 6), has had asthma for two years. This was preceded by sinusitis and running nose which began in September of 1951, three months after the birth of her first child. Between the ages of 7 and 12 years she had hay fever in the spring and fall.

The first attack of asthma began in June, 1952, early in her second pregnancy. This cleared in August, but then recurred in December, 1953, after delivery, and has been continuous since. Her eldest son has eczema.

Skin tests showed reactions to ragweed pollen and house dust extract and the patient has been treated with injections of these. Tests of pulmonary function have always been abnormal, but it is not clear at this time that irreversible changes are present. Weight, 118 pounds; height, 5 feet, 4½ inches.

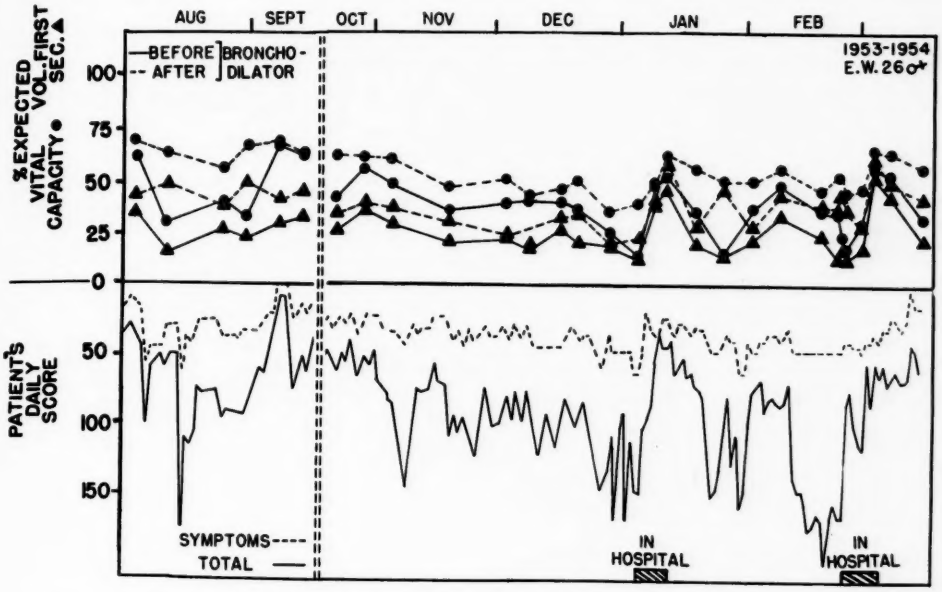

Fig. 5.—Respiratory studies and daily scores for Patient 4. (For explanation, see Fig. 2.)

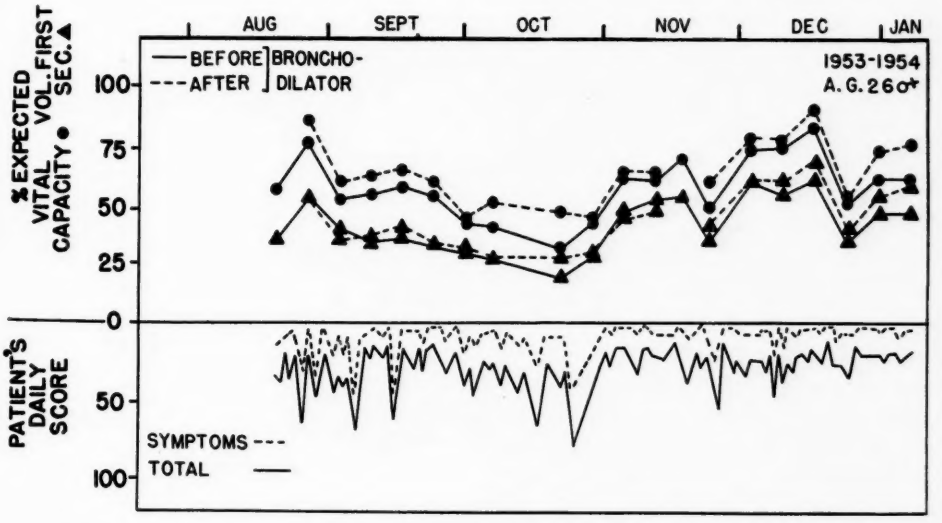

Fig. 6.—Respiratory studies and daily scores for Patient 5. (For explanation, see Fig. 2.)

RESULTS

The results obtained are shown in Figs. 2, 3, 4, 5, and 6. In the upper portion of each figure are the values of the vital capacity and the volume ex-

pired in the first second, obtained at roughly weekly intervals. These were repeated after six deep inhalations from a DeVilbiss No. 40 nebulizer containing isopropylarterenol in a concentration of 0.5 per cent. All the values shown are expressed as a percentage of the predicted normal.[4] The lower portion of each figure shows the results obtained with the score sheet; the solid line represents the total score, and the dotted line represents the score for symptoms. The difference between these two lines gives the score for medication.

DISCUSSION

In the three patients, W. K. (Fig. 2), J. E. (Fig. 3), and E. S. (Fig. 4), who received treatment with ACTH or cortisone and in whom the intensity of asthma varied widely, the daily scores correlated well with the changes in vital capacity and appear to give a reliable indication of the severity of bronchial asthma.

In the fourth patient, E. W. (Fig. 5), the correlation is less clear, but on the two occasions on which the patient was admitted to the hospital there was clear-cut improvement in that the requirement for medication decreased markedly and the measurements of pulmonary function became less abnormal. There has been no instance when pulmonary function in this patient has fallen within normal limits and we assume, therefore, as in the case of J. E. (Fig. 3), that some irreversible pulmonary disease (emphysema ?) is present. We have been struck by the large amount of medication taken by this patient, a factor perhaps in the lack of change in the tests of pulmonary function during most of the period of observation.

In A. G. (Fig. 6), relatively few changes are seen in either the daily score or the tests of pulmonary function. If the single set of measurements made in late December, 1953, is disregarded, then a clear difference is seen in both sets of values during December and January as compared with October. This correlated well with the general impression we obtained of the patient's state of well-being during the two periods, and we believe that the rather undramatic changes shown in the daily scores are nevertheless significant. This patient's illness appeared to vary in intensity over rather narrow limits and at no time did she appear to be either very ill or completely well.

In the case of W. K. (Fig. 2), while in the hospital and just before cortisone was given for the first time, the administration of bronchodilator drugs was purposely decreased because of the feeling that the patient was taking more medication than was warranted. There then occurred a prompt increase in the score for manifestations of asthma, and the spirometer measurements indicated a further aggravation of the asthma. We regard this as an illustration of the relationship which appears to exist in general between medication, manifestations, and pulmonary function among asthmatic patients. Although such a relationship is not surprising, we have been struck not only by the degree to which the taking of medication appears to reflect the severity of asthma, but also by the influence that medication, taken by the patient as needed, has on the vital capacity and the other measurements of pulmonary function. In

other words, the patient appears to take sufficient medication to maintain the level of pulmonary efficiency required for the avoidance of extreme discomfort. When medication taken at home fails to maintain this degree of pulmonary efficiency, the patient is forced to demand immediate medical aid. Since medication will influence pulmonary function, measurements of pulmonary function alone may not reflect the true severity of bronchial asthma, which will be appreciated only when medication, symptoms, and pulmonary function are considered together.

The daily score may be regarded as quantitative within certain limits. It appears to reflect changes in severity of symptoms adequately from day to day, but the data are entirely subjective and in the last analysis represent the patient's own concept of the severity of his disease. Therefore, such scores cannot be expected to give an indication of the relative severity of asthma in two different patients. What may appear to be a catastrophic illness to one may be borne with Spartan indifference by another. This is perhaps illustrated in Figs. 2 and 3. J. E. (Fig. 3) is a relatively stolid, phlegmatic person who complains little and appears to be relatively undisturbed by symptoms which, to his physician, appear severe. On the other hand, W. K. (Fig. 2) is a tense individual who has repeatedly expressed apprehension and has required considerable support. His scores have been distinctly greater than those of J. E. as regards both asthmatic manifestations and medication.

The possibility that apprehensive patients may overscore and therefore give a false indication of the severity of their disease cannot be disregarded. However, the same difficulty arises with other methods of assessing asthma where the patient's statement goes far to establish in the mind of the physician the degree of illness. For example, the patient is often seen during the day, at which time symptoms may be absent or minimal and the patient's description of cough, wheezing, orthopnea, and sleepless hours at night constitutes the only evidence that the patient has a significant amount of asthma. The daily score will give a numerical value to the patient's own evaluation of these symptoms under circumstances where memory is fresh.

SUMMARY AND CONCLUSIONS

Five patients with bronchial asthma were observed at intervals of one week or less over a period of approximately six months. Simple tests of pulmonary function were carried out at intervals of approximately one week or less. Each patient maintained a daily record of his medication, symptoms, and activities on specially prepared sheets. The data so obtained were converted to a numerical score as a measure of the intensity of asthma for each twenty-four-hour period. When this score was compared with the measurements of pulmonary function, the correlation was, for the most part, striking. The limitations of data obtained in this manner are discussed.

The results of this study indicate that no single criterion can serve as an adequate measure of the severity of bronchial asthma. A more reliable estimate is possible when the intensity of symptoms, the amount of medication required, and the results of simple tests of pulmonary function are taken into account.

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THE EFFECTS OF ACTH AND CORTISONE ON THE PATHOLOGY OF REVERSED ANAPHYLAXIS IN THE GUINEA PIG*

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THE advent of ACTH and cortisone has led to numerous studies on the effects of these hormones in various allergic states of man and in experimental types of hypersensitivity in animals. It has become clear that all forms of experimental allergy do not respond uniformly to those compounds. Differences in results depend upon the species of animal, the particular shock organ, the type and severity of the allergic reaction, the amounts and mode of administration of the hormones, and the time relationship between administration of the hormones and of the shock-eliciting material. One of these variants is illustrated by the fact that both ACTH and cortisone, in certain dosages, protected a definite percentage of guinea pigs from immediate fatal reversed and Forssman anaphylactic shock, but not from the usual direct fatal anaphylaxis.[1, 2]

Jervis[3] has reported that, following intracarotid injection of fatal doses of Forssman antibodies, pathologic changes were found in the corresponding hemisphere of the guinea pig's brain. It is known that reversed Forssman anaphylaxis is somewhat altered by ACTH and cortisone, and that pathologic changes in the central nervous system have been observed with this reaction. It is the purpose of this study to determine whether nonfatal reversed anaphylaxis of the guinea pig produced by intracardiac injection of Forssman antibodies is associated with demonstrable pathologic changes in the brain, and to investigate the effects of ACTH and cortisone on such lesions.

MATERIAL AND METHODS

Guinea pigs weighing between 250 and 350 grams were used throughout this study. For production of reversed Forssman anaphylaxis, a sheep cell (rabbit) antiserum (No. 61) (Forssman antibody) was prepared as previously described.[2, 3] This antiserum was injected intracardially to three groups of guinea pigs. A dosage was chosen to produce reversed anaphylactic shock without death in the majority of animals and was found to vary between 0.25 and 0.5 ml. in preliminary experiments. Group 1 (twenty-two animals) received this shocking dose of serum only; group 2 (ten animals) was treated with ACTH, and group 3 (nine animals) with cortisone, prior to this shocking dose. Two additional groups of guinea pigs served as controls and received

Presented at the Ninth Annual Meeting of the American Academy of Allergy, Boston, Massachusetts, February, 1953.

From the Children's Hospital of Buffalo.

Received for publication July 12, 1954.

*This investigation was made possible by a Grant-in-Aid from Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

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only the hormones. Group 4 (ten animals) received ACTH, and group 5 (eight animals) received cortisone.

The ACTH used was preparation 79R (Armour*) and was given subcutaneously in the amount of 0.5 mg. ACTH was dissolved just prior to its use. Cortisone-acetate (Merck†) was administered subcutaneously in amounts of 50 mg. Animals in groups 2 and 3 received the hormones twenty-four hours prior to the shocking dose of sheep cell antibodies.

The animals were observed closely for several hours after the administration of the sheep cell antiserum and then were inspected three times daily. Animals of each group were sacrificed (by intraperitoneal injection of Pentothal sodium) at different periods of time (up to sixty-four days) after the challenging dose of the antiserum. A few animals succumbed from either reversed Forssman shock or intercurrent infection during the observation period.

The guinea pigs used in the various experimental series were dissected immediately after they had been killed or when they were found dead in the cage. The brain, heart, lungs, kidney, liver, and spleen of all animals were removed for histologic examination.

The brain was studied after embedding in celloidin. Sections were taken from all areas, after the entire brain was dissected in four blocks in the coronal plane. The staining methods were Nissl, hematoxylin-eosin, and Trichrome (modification of Rasmussen). For lungs, heart, kidneys, liver, and spleen, paraffin technique was used with hematoxylin-eosin staining only.

The histologic examination of all specimens, which were identified by code numbers only, was completed by one of us (K. L. T.).

RESULTS

A summary of the data, described later in this article, is illustrated in Table I. In Group 1, one of the twenty-one guinea pigs died of intercurrent purulent meningitis and was excluded from further consideration. Twelve of the remaining guinea pigs had symptoms of reversed shock; one of them died from acute and two from delayed shock. The pertinent histologic findings are

TABLE I. REVERSED FORSSMAN ANAPHYLAXIS

	GROUP 1 UNTREATED	GROUP 2 TREATED WITH ACTH	GROUP 3 TREATED WITH CORTISONE
Total	20	10	8
Reverse shock	12	7	7
Fatal shock			
Acute	1	1	1
Delayed	2	0	2
Brain lesions			
Total	13/20*	2/10	5/8
1st to 15th day	9/9	1/4	4/4
16th to 64th day	4/11	1/6	1/4

*Numerator = Number of animals with brain lesions.
Denominator = Total number of animals.

*Armour ACTH (79R) was kindly supplied by The Armour Laboratories, Chicago, Illinois.
†Cortone Acetate was furnished through the courtesy of the Merck & Company, Inc., Rahway, New Jersey.

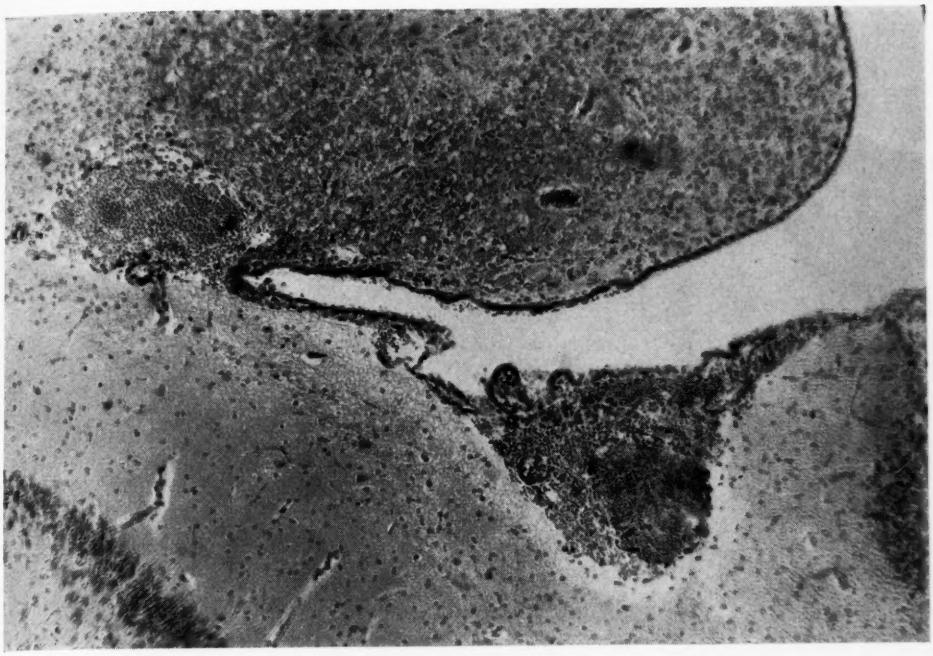

Fig. 1.—Note the marked congestion of the subependymal veins surrounded by hemorrhages (inferior horn of lateral ventrical).

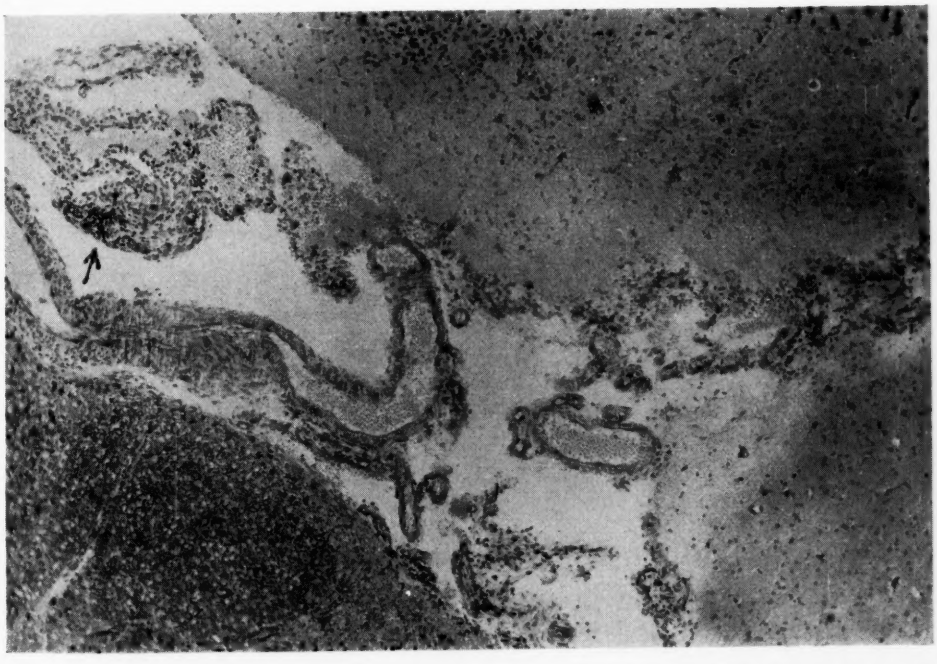

Fig. 2.—Note the meningeal hyperemia with very slight leukocytic infiltrations in the walls of the distended veins and marked leukocytosis within the lumina.

TABLE II. HISTOLOGIC FINDINGS IN GUINEA PIGS AFTER INJECTION OF FORSSMAN ANTIBODIES

ANIMAL NUMBER	DIED (D) OR KILLED (K)	TIME AFTER INJECTION	BRAIN	LUNG	KIDNEY	HEART
3	D	3 minutes	++	−	−	+
4	K	7 minutes	++	+	−	+
5	K	24 hours	++	+	−	+
6	K	2 days	++	+	−	++
11	D	19 hours	++	+	−	+
12	K	48 hours	+++	++	+	+
13	D	4 days	+	+	−	+
7	K	4 days	+++	+	+	+
22	D	12 days	meningitis	+++	−	+
8	K	14 days	+++	++	+	+
49	K	20 days	+	+	+	−
32	K	22 days	−	+	−	+
35	K	28 days	−	+	−	+
54	K	28 days	+++	+	+	+
43	K	30 days	−	+	−	+
59	K	34 days	−	−	−	−
44	K	41 days	+	−	−	+
55	K	49 days	+++	+	+	+
60	K	57 days	−	+	−	−
63	K	63 days	−	−	+	−
64	K	3 months	−	−	+	−

Brain
+ = Slight degree of hyperemia and occasional minimal hemorrhages.
++ = Distinct hyperemia, focal pericapillary hemorrhages, and slight focal inflammatory meningeal reaction.
+++ = Distinct encephalitic infiltrations in two animals; toxoplasma-like parasitic inclusions were observed either at or close to the site of the infiltration.

Other Organs
+ = Slight focal inflammatory infiltrations.
++ = Inflammatory changes of a more diffuse character.
+++ = Very marked bacterial infection.

presented in Table II. There is only one change which seems of some relative importance, and that is the finding of very marked hyperemia throughout the central nervous system, involving meninges and the nervous substance proper with capillary hemorrhages (Fig. 1) and with some, though very minimal, inflammatory reaction in the walls of some meningeal veins (Fig. 2) or, as in Case 6, in the wall of an artery (Fig. 3). In addition, there is minimal cellular inflammatory reaction in the meninges observed in one case (No. 11). In the animals which survived beyond fourteen days, this hyperemia is less marked (Table II).

TABLE III. HISTOLOGIC FINDINGS IN GUINEA PIGS AFTER INJECTION OF ACTH AND FORSSMAN ANTIBODIES

ANIMAL NUMBER	DIED (D) OR KILLED (K)	TIME AFTER INJECTION	BRAIN	LUNG	KIDNEY	HEART
15	D	10 minutes	−	−	+−	−
21	K	4 days	++	++	−	−
27	K	8 days	−	+	−	−
30	K	15 days	−	−	−	−
47	K	20 days	−	+++	−	−
36	K	22 days	−	+−	+−	−
41	K	28 days	−	−	−	+
52	K	28 days	−	−	−	−
56	K	31 days	+	−	−	−
61	K	41 days	−	++	−	++

For legend, see Table II.

In Group 2, ACTH (0.5 mg.) was injected intraperitoneally to each of ten guinea pigs twenty-four hours prior to a similar shocking dose of the same Forssman antiserum as was given to Group 1. Seven of the animals had symptoms of reversed shock, and one of these died ten minutes after the injection of the Forssman antibodies (Table I). The brains of two guinea pigs showed slight hyperemia of the meningeal veins, with hemorrhages. The brains of all other animals of this group were essentially normal (Table III).

In Group 3, nine guinea pigs each received 50 mg. of cortisone prior to the shocking dose of the same Forssman antiserum as was given to Groups 1 and 2. One animal was excluded because of death from intercurrent purulent meningitis. Seven of the remaining eight animals had symptoms of shock.

Fig. 3.—Distinct congestion of vein and artery with localized meningitic infiltration within and around the hyperemic vessel walls (in a sulcus above the corpus callosum).

One of these died from acute shock, and two from delayed shock (Table I). There were no significant pathologic changes in the brains of three of the eight animals. The brains of the remaining five guinea pigs showed slight vascular alterations similar to those in the untreated animals, although these findings were not as marked and widespread (Table IV).

In Group 4, a control group of ten animals each received 0.5 mg. of ACTH alone and were sacrificed at various times thereafter. One guinea pig died intercurrently from bacterial meningitis. Two of the nine animals showed slight pathologic changes in the brain (hyperemia and capillary hemorrhages). The brains of the remaining seven animals were normal (Table V).

TABLE IV. HISTOLOGIC FINDINGS IN GUINEA PIGS AFTER INJECTION OF CORTISONE AND FORSSMAN ANTIBODIES

ANIMAL NUMBER	DIED (D) OR KILLED (K)	TIME AFTER INJECTION	BRAIN	LUNG	KIDNEY	HEART
16	D	20 minutes	–	+–	–	–
18	D	3 days	+	+++	+–	+
39	D	8 days	+	+++	–	pericarditis
33	K	15 days	+	++	–	epicarditis
23	D	7 days	meningitis	+++	–	+–
25	D	14 days	meningitis	++	–	–
45	K	21 days	+	++	–	+ (epicarditis)
38	K	22 days	+	+	–	+ (epicarditis)
50	K	28 days	–	++	–	–
42	K	28 days	–	++	–	+–

For legend, see Table II.

TABLE V. HISTOLOGIC FINDINGS IN GUINEA PIGS AFTER INJECTION OF ACTH

ANIMAL NUMBER	DIED (D) OR KILLED (K)	TIME AFTER INJECTION (DAYS)	BRAIN	LUNG	KIDNEY	HEART
17	D	2	+	+++	–	+ (pericarditis)
20	K	4	+	–	–	–
26	D	8	meningitis	+++	–	+ (pericarditis)
31	K	15	–	++	–	–
48	K	20	–	++	+	–
37	K	22	–	–	–	+
40	K	28	–	+–	+	–
53	K	28	–	–	–	+
57	K	35	–	–	+	–
62	K	41	–	+	+–	–

For legend, see Table II.

In Group 5, the other control group of eight guinea pigs, each was given 50 mg. of cortisone. Two of these animals died from bacterial meningitis. Two of the remaining six animals showed slight hyperemia of the meninges and hemorrhages into the ventricle (Table VI).

TABLE VI. HISTOLOGIC FINDINGS IN GUINEA PIGS AFTER INJECTION OF CORTISONE

ANIMAL NUMBER	DIED (D) OR KILLED (K)	TIME AFTER INJECTION (DAYS)	BRAIN	LUNG	KIDNEY	HEART
19	K	3	+	+++	–	+
24	K	8	–	+	–	–
28	D	8	meningitis	+++	–	–
29	D	9	meningitis	+++	–	–
34	K	15	–	+++	–	+ (pericarditis)
46	K	21	+	++	–	–
51	K	28	–	++	–	–
58	K	35	–	+++	–	+

For legend, see Table II.

In addition to the pathologic findings of the brain described previously, another type of lesion was observed in five animals of Group 1 (animals 7, 8, 12, 54, and 55) (Table II). These histologic changes consisted of very distinct inflammatory reactions in various parts of the cortex and brain stem (Fig. 4). The leptomeninges above the cortex involved showed distinct inflammatory infiltrations. Since the relationship, if any, of these encephalitic lesions to

reversed Forssman anaphylaxis was unknown, it was deemed advisable to repeat the experiment. An additional twenty-five guinea pigs were injected intracardially with a different Forssman antiserum (No. 10) as in Group 1. In two of these twenty-five guinea pigs, lesions similar to those of the other five animals of Group 1 were found. Only after thorough search of the entire area of the inflammatory infiltrations and of the surrounding cortical tissue did it become apparent that these were parasitic inflammations. In two instances, either within or very near the infiltrations, accumulations of parasitic inclusions were found, strongly suggestive of toxoplasma (Fig. 4). These reactions appeared particularly recent in the animals killed within the first

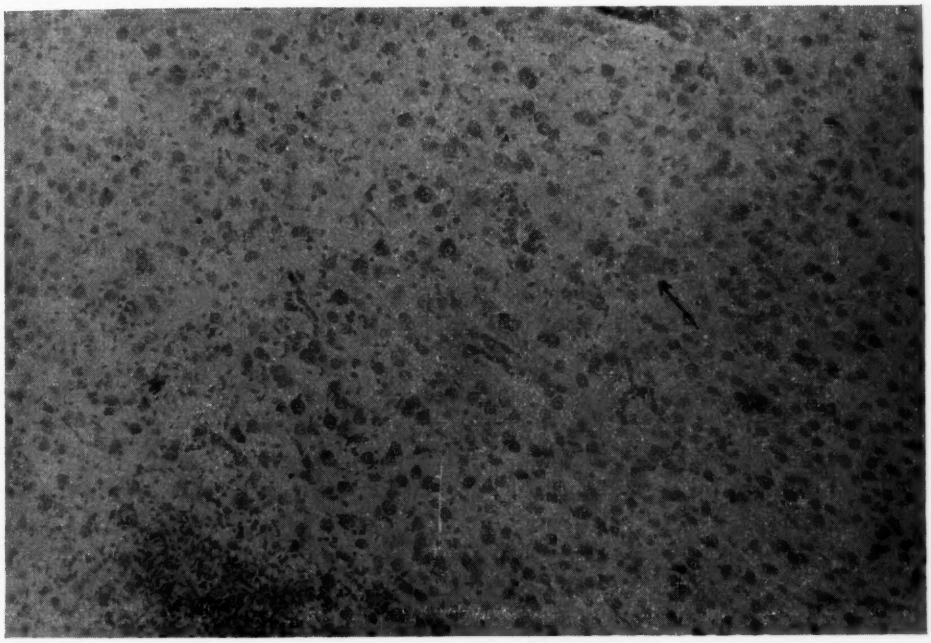

Fig. 4.—Typical encephalitic infiltration in the cortex. The pale area to the right of the center, especially marked, contains the toxoplasma-like parasites.

fourteen days. They also were present in a granulomatous state in an animal killed on the forty-ninth day. In one of the five killed on the twenty-eighth day these inflammatory changes in the cerebral cortex appeared nearly as recent as in the animal killed on the fourth day. We are obviously dealing, therefore, with a chance finding of a toxoplasma encephalitis in both series.* A comparison of the individual encephalitic lesions does not permit the conclusion that the experiment might have activated a silent parasitic infection in these seven animals. It is of interest, in this connection, that in all seven

*Regarding toxoplasmosis in animals, see

Markham, F. S.: Spontaneous Toxoplasma Encephalitis in the Guinea Pig, Am. J. Hyg. 26: 193, 1937.

Sabin, A. B., and Olitsky, P. K.: Toxoplasma and Obligate Intracellular Parasitism, Science 85: 336, 1937.

animals in which focal encephalitic lesions were present here were also some slight interstitial focal inflammatory infiltrations in the kidneys.

Only in the repeat experiment of twenty-five animals were the salivary glands regularly examined. Megalocytic inclusion bodies were found in the ducts of several animals, with no inflammatory changes in the central nervous system whatsoever. Only one of the two positive cases of the second series had some megalocytic inclusions, and in this case toxoplasma-like parasites were found close to a nerve cell within the inflammatory infiltrations.

It is evident from the tables that focal inflammatory changes in the heart muscle were frequently encountered, not only in the animals with parasitic brain changes but also in several others. Again, they were observed more frequently in the animals that had Forssman antibody only than in the hormone-treated Groups 2 and 3 and the control Groups 4 and 5 (Tables II to VI).

In every instance, liver and spleen were examined, but there were so few findings that were not entirely normal and largely related to the state of nutrition, particularly in the liver, that it is not pertinent to have them listed. There were no inflammatory changes present, as found in some of the other organs. A summary of the pertinent histologic findings in lungs, kidneys, and heart is shown in Tables II to VI.

DISCUSSION

It has been shown by the experiments just described that an intracardiac injection of a sublethal dose of Forssman antibodies produced lesions in the brain and meninges of guinea pigs. These changes were predominant in animals for a period of two weeks after the shocking dose and were less noticeable or absent in animals that were sacrificed at a later date. These lesions differed from those described by Jervis[3] in that he observed unilateral brain damage with demyelinization. There was no evidence of demyelinization in the experiments here reported. This may be attributed to the fact that in these studies an intracardiac, rather than an intracarotid, injection (Jervis) was employed. Also, in Jervis' work lethal doses of Forssman antibody were used, whereas in the present series of experiments sublethal doses were employed. Hence, either the route of administration or the dosage of Forssman antibody injected could explain the differences in the findings.

The site of localization of the reaction between the injected Forssman antibodies and the naturally present Forssman antigen in the tissues is apparently in the vascular system. It is here, in the capillaries and veins, that hyperemia and hemorrhages were noted in these experiments. Several other investigators have found that other types of allergic reactions (antigen and antibody union) may involve the vascular system. Rich, Berthrong, and Bennett[4] have shown this in their studies on lesions of periarteritis nodosa produced in rabbits sensitized to horse serum. Cohen and his associates[5] recently presented evidence that animals passively sensitized with streptococcus antitoxin show vascular lesions when shocked with streptococcus toxin. Also,

Rappaport and his co-workers[6] found changes in the blood vessels as well as in the ground substance and basement membranes of the epithelium of the nasal mucosa of patients with allergic states.

The present experiment revealed that ACTH and, to a lesser degree, cortisone decreased the pathologic changes of the vascular system of the brain. It is interesting to note that, according to Kabat and his associates,[7] cortisone (in proper dosage) will prevent the encephalomyelitis produced in monkeys by the injection of brain emulsion as the antigen, and others[8] have shown similar results in the brains of guinea pigs.

Similar beneficial effects from ACTH therapy have been noted in other vascular allergic lesions, for example, in experimental periarteritis nodosa[4] and in allergic rhinitis.[6] Furthermore, the Shwartzman phenomenon can be prevented by the administration of ACTH prior to the provocative, though not after the sensitizing, dose of antigen.[9, 10]

The effects of ACTH and cortisone on Forssman anaphylaxis of the guinea pig are paralleled by observation on other anaphylactoid reactions. Selye[11] reported that cortisone and purified ACTH inhibit the anaphylactoid reactions of the rat to the parenteral administration of egg white.

To explain the mode of action of these hormones on reversed Forssman anaphylaxis, three possibilities may be considered. (1) The beneficial effect could be due to the prevention of the reaction between Forssman antibodies and the homologous antigen present in the experimental animal. No evidence is available indicating that either ACTH or cortisone interferes with antigen-antibody reactions per se. (2) ACTH may prevent damage from the action of Forssman antibodies on the shock tissues in a nonspecific way. For example, Dougherty and Schneebeli[12] showed that traumatically induced inflammation, as well as allergic reactions, in mice similarly were inhibited by cortisone treatment. Likewise, Hoene and his associates[13] reported that ACTH effected a significant decrease in nonspecific "inflammatory potential" after injection of mustard suspension. It is conceivable, furthermore, that the Forssman antigen-antibody reaction in the blood vessels of the brains of guinea pigs may be followed by changes in permeability. (3) That ACTH and cortisone may alter capillary permeability nonspecifically was shown by Benditt and associates[14]; these authors observed that pretreatment for five days with either ACTH or cortisone largely inhibited the manifestations of increased capillary permeability due to the action of partially purified testicular hyaluronidase in rats. It may be interesting to determine whether hyaluronidase inhibitor alleviates the manifestations of Forssman anaphylaxis.

SUMMARY AND CONCLUSIONS

1. Distinct pathologic changes in the brain and meninges of guinea pigs are produced by the intracardiac injection of sublethal doses of anti-sheep cell (rabbit) antiserum (Forssman antibodies), consisting of marked hyperemia, capillary hemorrhages, and minimal inflammatory infiltrations in the meninges.

2. These lesions are ameliorated by a previous injection of ACTH in the scheduled dosages employed, and to a lesser extent by cortisone.

3. The exact mode of action of these hormones on this reaction is not explained, but several possibilities are discussed.

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SHORTCOMINGS OF THE EXPERIMENTAL METHODS FOR DETECTION OF SENSITIZING PROPERTIES OF NEW DRUGS

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ALLERGIC reactions are among the most frequent side effects associated with the use of modern drugs; there is hardly a medicament known that is entirely free of such properties. The cause of the large number of untoward reactions lies not only in the enormous increase in the use of drugs, but also in the fact that decidedly more powerful remedies have been introduced. Although allergic reactions are independent of the usual pharmacologic and toxicologic properties, it is, nevertheless, a regular observation that substances of greater chemical reactivity are stronger sensitizers and produce allergic side reactions more frequently than substances of low reactivity. There is little doubt that among the newer drugs there are more chemically reactive compounds than may be found among the lists of older medicinals.

In years not long past, a physician might have hesitated to prescribe such reactive substances, but present-day pharmaceuticals are safer from a purely toxicologic point of view, thanks primarily to the efforts of the Food and Drug Administration, which insists upon intensive experimental and clinical studies before it will countenance the commercial introduction of a new remedy in this country. Indeed, no preparation is released by this government agency before it has been adequately established that it is safe under the conditions for which its use is recommended in medical practice.

In order to increase still further the safety of drugs, it has been recently suggested by the Food and Drug Administration that, in addition to long-term toxicity studies, evidence be presented to show that a substance is free from major sensitizing properties before it can be officially released for medical use. The purpose of this article is to discuss the problem as to whether, with the aid of known techniques, we can ascertain experimentally that a given drug is nonallergenic, or whether it is possible, on the other hand, to demonstrate that a new substance is a strong sensitizer and, when used in medical practice, may produce an untoward number of severe allergic side reactions.

DRUG SENSITIZATIONS DURING MEDICATION AND EXPERIMENTAL REPRODUCTION

A. *Sensitizations Developing During Medication.*—The clinical symptoms of allergic side effects of drugs vary widely and may include not only known forms of allergic diseases but, in addition, many manifestations of still unknown pathophysiologic reactions. Allergy is a composite of numerous, very different

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Received for publication June 1, 1954.

reactivities. Consequently, before an analysis can be made of the various experimental procedures that are available for establishing the absence or presence of sensitizing properties, it is necessary to discuss briefly the characteristics of the two most important forms of allergic side reactions produced by drugs.

1. The sensitization of the mesodermal cell apparatus affects the cells of the capillaries and the bronchioli, as well as the smooth muscles of the intestines and glandular system. This is the *immediate* or *anaphylactic* type of sensitization, comprising the different types of atopic reactions.

Drugs endowed with an intrinsic power to frequently produce the immediate type of allergic side reactions are proteins, substances of high molecular weight, compounds which form colloidal solutions, and many simple compounds of low molecular weight which readily combine with proteins.

2. Another important form of allergic drug reaction involves the sensitization of the epidermal apparatus. The classical expression of this type of sensitization is the contact dermatitis and is most commonly designated as the *eczematous, contact,* or *delayed* type of sensitization. The shock organ is the epidermal cell; the clinical manifestations consist in erythema and acute, subacute, or chronic dermatitis. The contact type of sensitization is elicited in most cases by substances that reach the epidermis by contact from outside the body. However, many drugs produce this same type of reaction from within, after absorption through the stomach or after subcutaneous or intravenous injection.

Compounds most frequently responsible for the contact type of side reactions are simple chemical substances of low molecular weight. Many of them are chemically stable, others may acquire greater chemical reactivity after metabolic transformation within the body cells.

3. Certain authors distinguish the tuberculin type of sensitivity from the contact type. Although the tuberculin type is characteristic especially of the tuberculin hypersensitivity, similar or identical reactivities frequently occur during many other infections. Allergies against certain drugs, notably arsphenamines, may present the same form of reactivities, and are therefore considered as tuberculin type rather than as contact type of reactions. It is claimed that the shock organs involved in the tuberculin type of reaction are different from those concerned with the contact type of hypersensitivity. The principal site in the tuberculin type is the cutis, as in atopic sensitization, but at the same time the epidermis is strongly involved, as in the contact type. For this reason, the tuberculin type of reaction has been regarded as a combination of the immediate and the contact types of sensitization. However, this differentiation between the two very similar reactivities is by no means universally accepted, and the writer believes, with many colleagues, that both reactivities are identical. Certain histologic particularities which have been considered as specific for the tuberculin type of reactivity are not signs of an intrinsically distinct allergic effect, but rather are due to the particular nature of the eliciting agents.

The immediate and contact types of allergic side reactions to drugs seem to be based in every respect upon different processes. The antibodies involved, the allergotoxin released, and the manifestations produced are basically dissimilar.

As demonstrated by the variety of allergic side reactions, drugs differ in their allergenic characteristics. Penicillin, neoarsphenamine, nitro compounds, mercury salts, and such "biologicals" as antisera are strong sensitizers and each of these substances has produced a great number and a great diversity of allergies. Nevertheless, each of these substances produces preferentially *characteristic types* of allergic side reactions. Certain of these drugs, for instance, are responsible for allergic manifestations of the contact type primarily, while others are known to cause atopic symptoms, such as urticaria, asthma, or anaphylactic shock.

The aromatic nitro compounds and mercury salts, for instance, sensitize the epidermis almost exclusively, causing contact dermatitis and only very rarely atopic reactions, such as urticaria or asthma. Penicillin and tetanus antitoxin, on the other hand, generally produce allergic reactions of the atopic type, such as serum sickness, urticaria, and anaphylaxis, whereas the contact type of reaction is less frequent with penicillin and nonexistent, or at least very questionable, in the case of antiserum.

Sensitization to neoarsphenamine gives rise to two or three types of allergic reactions with almost equal frequency. Extremely severe cases of each of the various types have been observed, and fatalities have occurred after arsphenamine dermatitis and anaphylaxis as well.

In addition to these more common types of sensitization to drugs, there are many other forms which are neither accompanied by positive skin reactions nor experimentally reproducible in animals or man. Such reactions are lichenoid eruptions with Atabrine, fixed exanthemas with phenolphthalein, acneform eruptions with chlorinated compounds, purpura with arsenicals or gold preparations, and many others. We shall not include them in this discussion.

B. *Experimental Sensitizations.*—Numerous attempts have been made to experimentally reproduce these side reactions of drugs in animals and man, in order to study the conditions of their occurrence and the possibility of avoiding them.

Animals: Epidermal sensitizations of the contact type are elicited readily in guinea pigs after cutaneous or intradermal application of drugs known to be strong sensitizers for man, as, for example, primulin, poison ivy extract, aromatic amines such as p-phenylenediamine, nitro compounds, nitroso derivatives, picryl chloride, chlorobenzene, phenylhydrazine, and a number of others.

Anaphylactic sensitizations of guinea pigs have been actually induced with certain substances of low molecular weight, chemically combined with proteins. However, only a few attempts have been made to obtain this form of allergy with the simple chemical substances alone, in contrast to the numerous successful experiments to bring about sensitizations of the contact dermatitis type.

It has not been established, therefore, whether it is possible, even with special techniques, to produce anaphylactic reactivity regularly with nonproteinic material, including drugs known to produce atopic allergies in man.

One of the great difficulties encountered in experimental allergy research, especially when simple chemicals are used as antigens, is irregularity of results. A toxicologic or pharmacologic experiment is always reproducible, provided one uses proper doses and healthy animals. This is by no means the case with regard to allergic sensitization of animals, even if the strictest experimental conditions are maintained. Furthermore, it is not possible to imitate in animals the great variety of allergic reactions observed in man. Indeed, the experimental conditions under which anaphylactic and contact or delayed type sensitizations have been produced in animals have been at wide variance from those that prevail naturally when these same drugs sensitize man. In many instances there seems to be no distinct correlation between experimental allergies and those that occur naturally in man.

In all animal experiments on sensitizations, numerous factors, independent of the allergic process itself, influence the result. The degree of sensitization may suddenly decrease or increase; often, nutritional and genetic factors may strongly interfere. I have often found, as have many other investigators, that recognized strong sensitizers of man may fail to produce a single case of sensitization in animals. This is generally the rule in all attempts to produce atopic sensitizations experimentally with simple chemicals of low molecular weight and low reactivity which are known to produce urticaria, fixed exanthema, or asthma in man. The failure to reproduce, with drugs, atopic reactions in guinea pigs is one of the arguments in favor of the separation of atopy and anaphylaxis.

Experimental sensitizations in man: It was an understandable and natural move to study experimental sensitizations in man. Various substances have been used for this purpose, especially those which were known to sensitize man under normal exposure. Most regularly successful, as with guinea pigs, were sensitizations of the contact type of hypersensitivity. Primulin, phenylhydrazine, poison ivy extract, p-phenylenediamine, picryl chloride, chlorobenzene, nitroso compounds, and arsphenamine, for instance, are strong experimental sensitizers for human skin. However, as in experimental animals, all attempts to produce experimental atopic sensitizations in man with simple chemical compounds have failed.

C. *Tests for Allergic Potentialities of New Drugs.*—The foregoing discussion on facts in experimental allergy points to several conclusions.

1. It is not correct to speak without qualification of *the* sensitizing property of a substance. The term *allergic sensitization* includes a number of very different forms of allergies, and drugs are capable of producing any one of a number of allergies. Consequently, a study of the allergic potentialities of a new drug should consider the various forms of sensitization. Before declaring that a drug is safe from the allergy point of view, one should determine whether or not it produces an atopic or a contact or delayed type of sensitization. In

most instances the nature of the drug and its proposed therapeutic use determine which allergic type of side reaction may be expected to occur more frequently. An antibiotic, for instance, which is injected is much more likely to produce severe atopic reactions, including dangerous states of anaphylaxis, than a drug used as an external disinfectant. If a substance used topically is a strong antigen, we can expect that it will produce contact dermatitis, rather than urticaria or asthma.

2. It is possible to sensitize animals with simple, chemically defined drugs. The most regularly obtained sensitization is contact dermatitis; other forms of allergy, especally the atopic type, have been successfully produced in only a few instances and, in most cases, the results obtained were irregular.

Test for allergenicity of new drugs in animals: Under these circumstances the question arises as to whether the available methods for producing experimental sensitization in animals are sufficiently reliable for ascribing to given compounds the ability to produce, in man, one or another type of sensitization, or for declaring a drug safe and free of major sensitizing properties.

In the writer's opinion, this is not the case. Only those experimental sensitizations of animals that produce allergies of the contact type succeed with sufficient regularity to permit a tentative conclusion referable to the possible behavior in man of the drug in question. If a substance sensitizes the skin of guinea pigs readily and strongly, then it may be inferred that it is not proper to use this compound as an external remedy. One is not justified, however, in concluding that this substance is suspect with respect to its ability to produce a large number of untoward allergic symptoms when administered by mouth.

It must be emphasized that even the relatively reliable method of studying sensitization of the epidermal apparatus is by no means foolproof. Substances innocuous for guinea pigs in the usual form of experimental application may be active allergens in man, when used in an entirely different form, vehicle, or manner of administration. On the other hand, I have observed 100 per cent positive eczematous skin reactions in guinea pigs with substances which in man produced very few skin allergies when administered by mouth, in spite of prolonged use. It is essential, therefore, to bear in mind that the conditions of the guinea pig experiment are not always identical with those prevailing in human therapy.

With regard to the other types of sensitization, especially the serious anaphylactic type of reaction, we do not have any experimental test method that will furnish evidence that any given substance of low molecular weight, such as the majority of our drugs, is capable of producing allergic side reactions of the atopic type, including anaphylactic shock.

We conclude, from the foregoing discussions, that none of the established methods of animal sensitization permits an adequate assessment of the potential sensitizing power of new drugs. Even the most reliable of these procedures, namely, the skin sensitization test of guinea pigs involving direct contact, is not sufficiently adequate to form the basis of a decisive official test.

Test for allergenicity of new drugs in man: It has been proposed that, instead of animals, human beings be used as subjects for sensitization tests. It seems likely that man is more readily and more regularly sensitized than guinea pigs and, furthermore, it is conceivable that experimental sensitization in man would yield more pertinent information than experiments in animals, since man is a natural victim of drug sensitizations. Indeed, practically all persons can be sensitized to strong sensitizers such as primulin,[2] p-phenylenediamine,[9-11] or poison ivy,[1] and approximately 80 per cent of the population can be rendered sensitive to 2,4-dinitrochlorophenol and related compounds.

The question therefore arises as to whether we should subject human volunteers to experimental sensitizations of any type for the purpose of an official hypersensitivity test of new drugs.

Experience has shown that the experimental sensitization of individuals to any substance with which they may subsequently come in contact or use therapeutically is fraught with considerable danger. We know of human volunteers who had been experimentally sensitized to generally used medicaments and who later, when they became ill, received these same drugs as part of their therapy. They promptly reacted with severe manifestations of hypersensitivity, and sudden death has occurred in a few instances. In the writer's opinion, it is unwise to sensitize human beings experimentally, not only to substances that are likely to be used as remedies but also to other chemicals not currently intended for medical purposes. It has been proposed to use aromatic amines or nitro compounds, for instance, for experimental sensitizations of man, substances which also have been widely used in animal sensitizations. Can we do this with a clear conscience? It is proved that from the very outset, or very rapidly after it has been established, any skin sensitization extends to many chemically related substances (cross sensitization). Numerous important and unexpected cross sensitizations in the group of aromatic amines and nitro compounds are well known,[9-12] and many of the substances included in the cross reaction to aromatic amines are frequently used as dyes for fabrics, leather, foods, and cosmetics. We are in daily contact with numerous compounds of this group and, furthermore, many of the aromatic amines or nitro compounds are, or form the base of, a variety of widely used medicaments. Assuming that a substance is chosen for experimental sensitization which is not currently used as a drug (for example, dinitrochlorobenzene or picryl chloride), we do not fear danger of cross sensitization. However, we must bear in mind that such a menace may arise unexpectedly at any moment. The voluntarily sensitized person is endangered for the rest of his life, since it is well known that natural and artificial sensitizations persist for long periods and probably for the duration of life.

A number of attempts actually have been made in the past to sensitize human beings for experimental purposes but this by no means implies that all such experiments can be justified. In some instances the sensitizing agent for man was chosen with great discrimination. Biberstein,[1] for example, selected poison ivy as the sensitizing agent for his Silesian volunteers; he could use this substance with a clear conscience, since he knew that poison ivy is nonexistent

in Europe and the probability that any of his patients would settle in the United States or handle Japanese lacquered boxes was at that time rather remote. In this country, any attempt at experimental sensitization of human beings to poison ivy would be highly undesirable.

CONCLUSIONS

This survey indicates that we are not in a position at the present time to experimentally prove or disprove with sufficient certitude that an unknown compound may produce an objectional number of major allergic reactions when used as a remedy. The conditions of an experimental sensitization and of a normal therapeutic exposure are entirely different. It would be unwarranted to condemn a new compound on the grounds that it is capable of sensitizing the skin of guinea pigs when injected intracutaneously, or the skin of man when applied for a number of days in the form of a "prophetical" patch test. Although such a compound would probably produce allergic skin reactions of the contact dermatitis type when used in sufficiently high concentrations in the form of an ointment, it may well be used without producing allergic side effects as an orally administered drug. If a prospective remedy is pharmacologically so active that small doses given within a short period of time suffice to produce a therapeutic effect, then the danger of sensitization becomes small, even with an allergenically very potent substance.

On the other hand, we do not know of any experimental procedures in animals or man which would indicate with sufficient certitude that a new, simple, chemical substance may produce atopic anaphylactic sensitizations in man. There can be no doubt that anaphylactic side reactions, especially anaphylactic shock, are more dreaded than any other form of allergic skin reaction. Thus far, only clinical trial will show whether a compound possesses the intrinsic power to produce atopic side reactions. The clinical trial alone will indicate, furthermore, whether such side effects, if they occur, are frequent or severe, and whether they represent a contraindication for its use, thereby precluding the release of the compound as a remedy. We must consider that, in many instances, the therapeutic usefulness may outweigh the importance of any allergic side effects. This is the case with most antibiotics and many other chemotherapeutic agents.

What should we do in view of this discouraging situation? We should, by all means, continue to evaluate in animals (and not in man) the skin-sensitizing properties of new compounds, but we must be very careful in our conclusions; we should not, a priori, stop clinical trials with powerful drugs if they produce strong sensitizations of animals. We must consider positive results of sensitizing experiments only as a warning to proceed with special care in clinical assays.

It is most important to pursue systematic studies of experimental sensitizations, especially those which pertain to the atopic allergies following the use of simple chemical compounds. There are many experimental approaches and, as long as such experiments are carried out in animals, the entire arsenal of

allergenic chemical and therapeutic agents may be employed to produce sensitizations. Since, however, the gap between human atopy produced by simple chemicals and experimental production of the same atopies in animals has not been bridged (unless special expedients are used, such as tubercle bacilli and other adjuvants), we also may resort to experimental sensitizations in human beings. In these cases, however, we should confine experimental sensitizations to allergens with which the population of this country has little, if any, contact. Such a substance, for instance, is the allergen derived from the highly skin-sensitizing *Primula obconica,* the "birthday gift" plant in Europe which is hardly used here. Regarding substances known to produce atopic sensitizations, it seems, at least in this country, that the arsphenamines are gradually disappearing as therapeutic agents, although it still may be too early to recommend them freely for experimental sensitization studies in man. One should be very careful in sensitizing human beings to nitro compounds and other substances that are members of that immense realm of cross sensitizations known as allergy to compounds of quinone structure.[11]

Much work is necessary in devising methods that will permit the assessment, in a routine manner, of the allergenic propensities of a new drug intended for human medication.

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THE USE OF CORTISONE OR ACTH DURING RAPID DESENSITIZATION OF ALLERGIC PATIENTS

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THE studies reported here were initiated in June, 1950. A patient with diabetes insipidus had developed clinical manifestations of anaphylactic hypersensitivity following treatment with posterior pituitary substances. With the aid of cortisone, the allergic symptoms were controlled and held in abeyance during a period of rapid desensitization with Pitressin tannate in oil. It was possible within a relatively short period of time to discontinue cortisone therapy and proceed with the required amounts of Pitressin without further allergic reactions to the substance. Encouraged by the therapeutic results of this first patient and the accompanying implication that cortisone had not interfered with the mechanisms of desensitization, attention was directed to the hypothesis that certain patients with hay fever, bronchial asthma, and allergic eczema might be managed more expeditiously by employing a similar technique of treatment.

Harvey and his co-workers[1-5] and others[6-11] have demonstrated the beneficial effects of ACTH and cortisone in a wide variety of clinical manifestations of hypersensitivity. Likewise, Rich and his group[12-15] have shown experimentally in animals that ACTH and cortisone exhibit an inhibitory effect upon the development of periarteritis nodosa, cardiovascular inflammatory lesions, and acute glomerulonephritis resulting from hypersensitivity.

Loveless[16] studied the effect of ACTH on sensitizing and blocking antibodies in one patient with perennial atopic dermatitis with summer exacerbations, who gave intradermal reactions to pollens of trees, grasses, and ragweed, as well as to dog and horse dander. This patient was first given ACTH therapy; consequently, there was an increase in conjunctival tolerance to timothy pollen, ragweed pollen, and horse dander. While on ACTH therapy alone, it was demonstrated that the circulating skin-sensitizing antibodies remained unchanged; moreover, there was no evidence for the presence of blocking antibodies. The patient, while still taking ACTH, was then given a large dose of specific antigen (first timothy, and later ragweed and horse dander). The author accordingly demonstrated that blocking antibodies were produced to timothy pollen but not, for some unexplained reason, to ragweed and horse dander.

The opinions and views set forth in this article are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

Presented at the Annual Meeting of the American Academy of Allergy, Feb. 26, 1953, Boston, Mass.

The ACTH used in this study was supplied by the Armour Laboratories and E. R. Squibb & Sons.

Received for publication March 8, 1954.

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While our studies were being carried out to learn whether or not it was possible to rapidly desensitize patients with the inherited type of hypersensitivity during treatment with ACTH or cortisone, an opportunity was afforded to treat similarly another patient with the acquired type of hypersensitivity. The patient, a diabetic, had developed an allergy to all forms of insulin.

METHODS

There were sixty-six patients* in the study, eight of whom had the acquired type and fifty-eight the hereditary type of hypersensitivity.

Of the patients with the acquired type of hypersensitivity, one was sensitive to posterior pituitary substances (Case 1), one to insulin (Case 2), two to tetanus antitoxin, and four to tetanus toxoid. With the exception of one patient who was sensitive to tetanus toxoid and who received ACTH during desensitization therapy, the other seven patients were given cortisone orally during desensitization. The two patients sensitive to tetanus antitoxin had previously had an anaphylactic reaction to an injection of this substance. These two patients were given 200 mg. of cortisone within the twelve hours prior to desensitization and 400 mg. of cortisone during the course of desensitization therapy. Desensitization was carried out by starting with the lowest dilution to which they did not give a positive intradermal reaction (that is, if they reacted to 0.1 c.c. of a 1-1,000 dilution of tetanus antitoxin, then desensitization was started with 0.1 c.c. of a 1-10,000 dilution). Desensitizing doses were given in gradually increasing amounts at intervals of every twenty minutes. Treatment was completed in about fourteen hours, during which time one patient received a total of 13,550 units and the other 50,000 units of tetanus antitoxin. Of the four patients who gave significantly positive skin test reactions to tetanus toxoid, three received only two doses of cortisone during desensitization (100 mg. the night before and 100 mg. the morning of desensitization). One patient desensitized to tetanus toxoid received 15 mg. of ACTH at the time of the first desensitizing dose, 15 mg. halfway through treatment, and 15 mg. at the completion of treatment. All four patients began with an initial dose of 0.1 c.c. of a 1-10 dilution of the toxoid and gradually increasing doses were administered at fifteen- to thirty-minute intervals until the total immunizing dose had been received. The time for completion of desensitization in all was three to four hours. With the exception of one of the four patients in this group, who had transitory urticaria during the course of desensitization with tetanus toxoid, there were no untoward reactions.

There were fifty-eight patients with the hereditary type of hypersensitivity. Their ages ranged from 2 to 71 years, with an average of 27.5 years. They were equally divided as to sex; twenty-nine were male and twenty-nine were female. Twenty-seven had both bronchial asthma and rhinitis; two had asthma, rhinitis, and eczema; three had asthma and eczema; three had rhinitis and eczema; ten had asthma alone; eight had rhinitis alone; four had eczema

*The patients with diabetes insipidus and mellitus, Cases 1 and 2, were seen at the U. S. Naval Hospital, San Diego. All other patients were from the private practice of E. L. K. and C. F. M.

alone; and one had vernal conjunctivitis. Of the thirty-two patients who had asthma, either alone or in combination with one of the other forms of hypersensitivity, there was an associated upper or lower respiratory tract infection in twenty-two instances. It was not possible to be entirely sure of the average duration of symptoms for this group of fifty-eight patients, as not all of them could state definitely when their symptoms first occurred. The stated duration of symptoms varied from three months to forty-seven years.

The patients with the hereditary type of hypersensitivity were desensitized with extracts of pollens, environmentals, and vaccines (stock, autogenous, or a mixture). The term *environmentals* applies to extracts made from dust, feathers, animal danders, etc. The pollen extracts were standardized so that 1 c.c. of concentrated solution contained, depending upon the pollen, from 25,000 to 30,000 protein nitrogen units. Environmental extracts contained, depending on type of environmental, from 5,000 to 10,000 protein nitrogen units per cubic centimeter of concentrated solution. The concentrated vaccines contained 8 to 10 billion organisms per cubic centimeter, depending upon the organism used. Desensitization therapy was given from Monday through Saturday, but never more than once on Saturday and not at all on Sunday, with the exception of five patients who were treated three times a day, seven days a week, while in the hospital.

Depending upon the degree of skin sensitivity to intradermal tests, patients were started on desensitization with dilutions ranging from 1-1,000,000 to 1-10,000. An initial dose of 0.1 c.c. of the highest dilution was followed by doses increasing by 0.05 to 0.15 c.c. until a dose of 0.8 c.c. of that dilution had been reached. Then 0.1 c.c. of the next lowest dilution was given and the same schedule followed until the optimum concentration was reached.

Nine patients received treatment once a day; thirty-three, twice a day; twelve, three times a day; and four patients were irregular in their treatment schedule. These latter four patients only occasionally lapsed from treatment for as long as three days.

Concomitant with desensitization therapy, forty-eight patients were given ACTH intramuscularly alone; two received both ACTH and cortisone, but not simultaneously; and eight patients were given only cortisone. Thirty of the fifty patients who received ACTH were given this hormone once a day; fourteen, twice a day; and six were given it three times a day. The total daily dose of ACTH was 10 mg. for three, 15 mg. for six, 20 mg. for two, and 30 mg. for three patients. Fourteen patients received 10 international units daily; one, 15 units; twenty, 20 units; and one, 30 units.* Of the fourteen patients treated with ACTH in milligram dosages, the individual total dosage ranged from 160 to 600 mg. The average total dose for this group was 338 mg. Of the thirty-six patients who received ACTH in international unit dosages, the individual total dosage ranged from 180 to 1,165 units. The average total dose per patient was 413 international units. The total duration of treatment with ACTH ranged from ten to seventy-one days, with an average of twenty-five days. Of the eight patients who were given rapid desensitization with the help of cortisone,

*An international unit of ACTH is equal to 1 mg. of original Armour LA-1-A.

the total days of treatment varied from twelve to fifty-five days, with an average of thirty-one days. The lowest daily dose given any one patient was 25 mg. for ten days, followed by 12.5 mg. for twelve days. The largest dose was 200 mg. for one day, followed by 100 mg. for twelve days. The total dosage of cortisone varied between 400 and 3,600 mg., and the average dose was 1,826 mg. Of these fifty-eight patients with the hereditary type of hypersensitivity, fourteen have been followed for more than twelve months; three have been followed from nine to twelve months; five from six to nine months; ten from three to six months; seven from one to three months; and nineteen have not returned for follow-up.

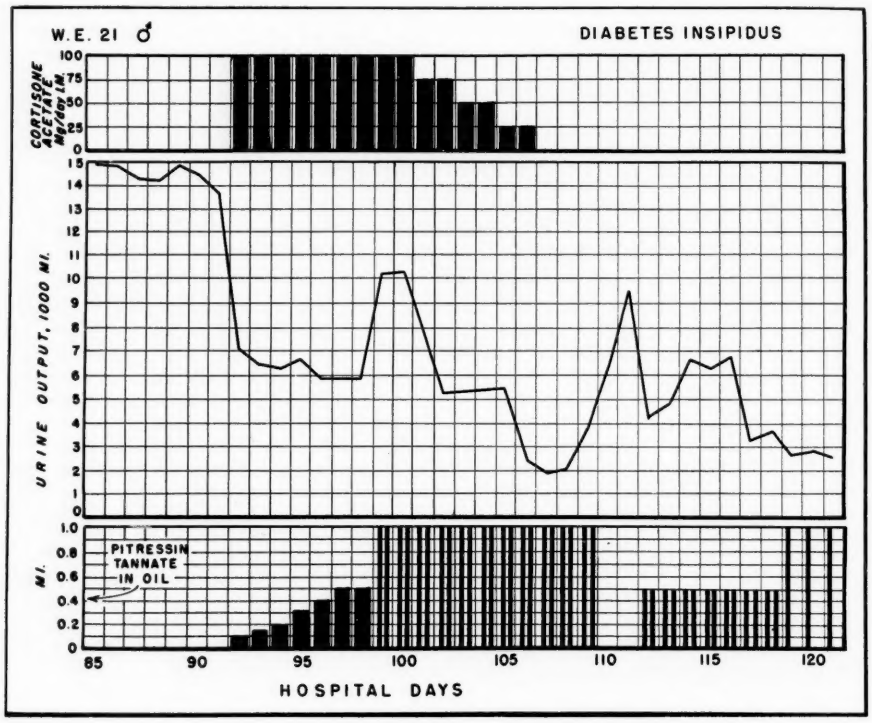

Fig. 1.

CASE REPORTS

Two cases of the acquired type of hypersensitivity are briefly described.

Case 1. W. E. Anaphylactic Reaction From Posterior Pituitary Substances (Fig. 1).—The patient was, at the time of examination, a 21-year-old man, who, prior to March, 1950, had been in good health and had not experienced any unusual infections or injuries. His family history was noncontributory. He was admitted to the U. S. Naval Hospital, San Diego, on April 18, 1950, with the complaint of increased thirst and copious excretion of urine. A complete physical examination was well within normal limits. His twenty-four-hour urine output was 15 to 20 liters. The specific gravity of the urine was 1.001 to 1.003. Except for the low specific gravity, urinalysis was negative. Other laboratory examinations as well

as x-ray examinations, which included skull films, were reported as normal. On May 8, 1950, treatment was started with obstetrical Pituitrin, 1.0 c.c. (20 units) twice daily, with a resultant decrease in the urinary output from 15 to 7 liters per day. Pitressin tannate in oil was then substituted for Pituitrin and the fluid intake and output continued at about 7 liters per day. On May 26 posterior pituitary powder by nasal insufflation was substituted for Pitressin tannate in oil, with a resultant drop of fluid intake and output to 2 to 3 liters per day. Two days after insufflation was begun (May 28), he developed symptoms of rhinitis and for this reason the nasal insufflation was discontinued. Treatment with Pitressin tannate in oil was reinstituted and immediately following the injection of 2 c.c. he developed headache, nausea, vomiting, generalized urticaria, and severe asthma. Treatment with posterior pituitary powder by nasal insufflation was tried again, but immediately this was followed by severe rhinitis, diffuse and generalized urticaria, and severe asthma. He was referred to the allergy clinic where intradermal skin tests were performed with 0.05 c.c. of a 1-1,000 dilution of Pituitrin, Pitressin, and Pitocin, and extracts of pork and beef. Huge reactions were elicited to Pituitrin and Pitressin; a mild reaction to pork; and negative reactions were obtained to Pitocin and beef. Scratch tests were then performed with posterior pituitary powder, Pitressin tannate in oil, and peanut and sesame oils. Large reactions were obtained to posterior pituitary powder and Pitressin tannate in oil. The reactions to peanut and sesame oils were negative. The passive transfer of antibodies was demonstrated to Pitressin and Pituitrin, but not to Pitocin. The patient was then rapidly desensitized to Pitressin tannate in oil under the protective shield of cortisone. During the first six days he was given 100 mg. of cortisone, intramuscularly, daily; and concomitantly the dosages of Pitressin tannate in oil were increased from 0.1 c.c. four times daily to 0.5 c.c. four times daily. During the subsequent days, the dosage of Pitressin tannate in oil was changed to 1 c.c. twice daily and the dosage of cortisone was decreased 25 mg. every third day. The patient received a total of 1.2 Gm. of cortisone over a period of fifteen days. Because of a retention of fluid and a decrease in the daily urine output on the seventeenth day, Pitressin tannate in oil was discontinued for two days and then reinstituted at a dosage of 0.5 c.c. twice daily for one week. Thereafter, 1 c.c. was given each day. The patient has been maintained on this latter dosage for the past two and one-half years without the use of cortisone and with a urinary output ranging between 3 and 4 liters a day. There has been no recurrence of his allergic symptoms since he was desensitized.

Case 2. E. B. Anaphylactic Reaction From Insulin (Fig. 2).—This 69-year-old patient, a known diabetic since 1922, had been reasonably well controlled on a 1,800 calorie diet. In 1939 he was given regular insulin to facilitate the management of his diabetes, but after the third dose he developed urticaria and angioneurotic edema. Further insulin therapy was at that time considered inadvisable. In 1949 another attempt was made to treat him with insulin; however, because of the knowledge of his former experience with insulin, he was given only 4 units of regular insulin. Immediately following this dose he developed angioneurotic edema and went into shock. In August, 1951, he was admitted to the U. S. Naval Hospital, San Diego, with a paronychial infection of the left great toe. At that time he was treated, to no avail, with antibiotics, surgical drainage, and soaks. Because his diabetes was getting out of control, insulin therapy was again reconsidered. However, before insulin therapy was reinstituted he was referred to the allergy clinic for consultation. Positive reactions were obtained to the intradermal administration of 0.01 unit of regular, protamine zinc, globulin zinc, and N.P.H. insulins. The passive transfer of antibodies was demonstrated to all of the above forms of insulin. Intradermal skin tests to extracts of pork and beef were negative. It was decided to attempt to desensitize the patient with regular insulin along with the concomitant administration of cortisone. In preparation for desensitization, 1-10, 1-100, and 1-1,000 dilutions of insulin, U.S.P. 100 units per cubic centimeter, were prepared. Desensitization treatment was then initiated with 0.1 c.c. of a 1-1,000 dilution of regular insulin, which was the equivalent of 0.01 unit of insulin. Treatment was given every four hours from 8 A.M. to 8 P.M. in increments of 0.1 c.c. When the

dosage of 1 c.c. of a 1-1,000 dilution was attained, treatment with the next lower dilution was initiated and carried out in an identical manner. When the dosage of 1 c.c. of a 1-10 dilution was attained, being equivalent to 10 units of insulin, then 10 units of undiluted insulin (U.S.P. 40 units per cubic centimeter) were administered morning and night. The daily dosage of insulin then fluctuated until it was determined that his disease could be controlled with 15 units of regular insulin once daily. During the first eight days of insulin desensitization therapy, cortisone was administered orally in a dosage of 50 mg. every twelve hours. For the next ten days 100 mg. was given orally every twelve hours, and for the ensuing eighteen days the dosage was gradually reduced. It was possible, thereafter,

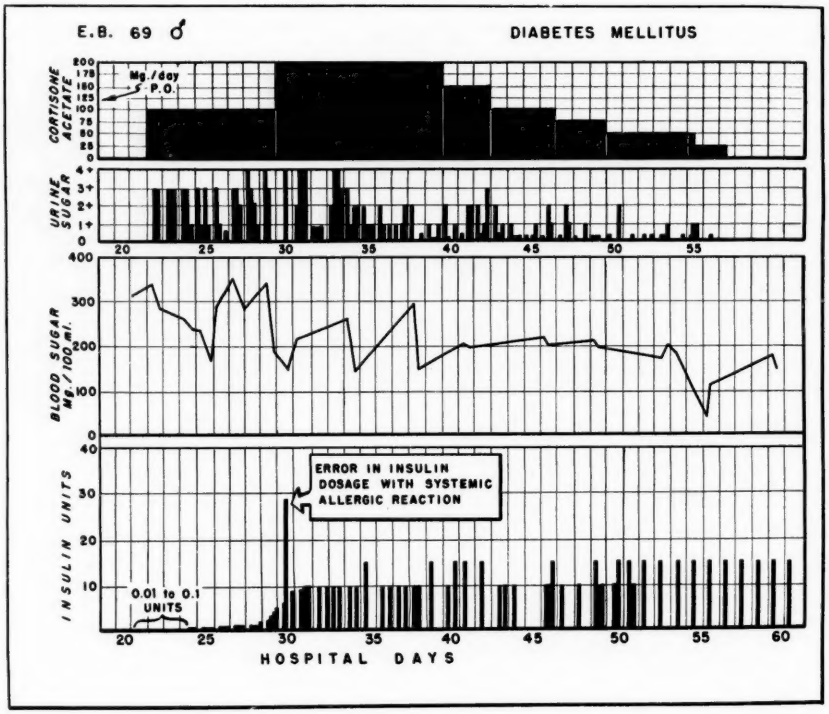

Fig. 2.

to give the patient 15 units of insulin each morning without further cortisone therapy, and his diabetes remained under control. In all, he received a total of 4.2 Gm. of cortisone over a period of thirty-six days. He was followed for a year and a half, during which time he had no further allergic reactions from insulin.

RESULTS

Rapid desensitization, combined with the concomitant administration of ACTH or cortisone, was successfully carried out in eight patients with the acquired type of anaphylactic hypersensitivity. This is evidenced by the fact that the patients hypersensitive to Pitressin (Case 1) and to insulin (Case 2), respectively, were able subsequently to receive, without the aid of cortisone, adequate therapeutic doses of their required hormones without accompanying allergic reactions. Two patients who, prior to rapid desensitization, had

experienced severe anaphylactic reactions to tetanus antitoxin could take full protective doses of tetanus antitoxin without untoward reaction when desensitization therapy was combined with simultaneous cortisone therapy. Four patients who, prior to rapid desensitization with the aid of hormone therapy, had positive skin test reactions to tetanus toxoid were given full protective doses without severe immediate or subsequent local or constitutional allergic reactions. It is granted that before the advent of ACTH or cortisone attempts were made to desensitize patients with manifestations of hypersensitivity similar to these eight patients, but not always successfully and with some risk on the part of the patient and considerable anxiety on the part of the physician.

All of the fifty-eight patients who had the hereditary type of hypersensitivity were having active symptoms of their various types of hypersensitivity at the time rapid desensitization and hormone therapy were begun. All fifty-eight patients were able to complete rapid desensitization in an average of about three weeks. During the course of rapid desensitization there was, in all instances, improvement or relief of symptoms, due in all probability to the effect of ACTH or cortisone. There were no serious treatment reactions in any of the fifty-eight patients. There were occasional local reactions; however, no more than would be anticipated in a similar group of patients receiving routine desensitization therapy with the same type of extracts. On some occasions it became necessary to drop back on the dosage of one or the other of the extracts and to increase again or to hold the dose for one or two days.

It is imperative to emphasize clearly that data so obtained, not only for this group of patients but for any chronic disease, are most difficult to assay, and it must be recognized that an accurate estimation of clinical improvement in patients with allergic rhinitis, bronchial asthma, and allergic intrinsic eczema are virtually impossible because of the number of clearly variable factors involved.

DISCUSSION

It has become increasingly evident that the field of greatest therapeutic promise for the two hormones, cortisone and ACTH, resides in the so-called collagen diseases and a variety of the allergic states. Although these substances alleviate the clinical manifestations of allergic rhinitis, bronchial asthma, and urticaria, such symptoms invariably recur in the same shock tissues upon withdrawal of the hormones. Therefore, the practical therapeutic usefulness of these hormones in the management of such protracted disabilities is limited. Irrespective of this, ACTH and cortisone are still two of the most effective agents for the symptomatic treatment of allergic diseases, acute or chronic as the case might be. The present study suggests another use for these hormones, which achieves practicability by combining their administration with rapid desensitization.

It is not suggested that the combined therapy of rapid desensitization and cortisone or ACTH be given to all patients with active symptoms of hypersensitivity, but merely when the proper circumstances arise. Cases 1 and 2 are examples of the invaluable aid given by cortisone in the desensitization

of two patients who were in desperate need of another type of hormone therapy to control their respective diseases. The results obtained in the management of these two patients represent our only justifiable evidence that cortisone and ACTH do not interfere with the mechanisms of desensitization.

It is an accepted fact that desensitization therapy is important in the control of bronchial asthma, hay fever, and certain types of eczema. The favorable clinical effects of routine desensitization are usually delayed and ordinarily do not appear until four to eight months following the onset of treatment. The present studies suggest that the same beneficial effects might be obtained in a much shorter period of time. The primary limiting factor to rapid desensitization would seem to be the difficulty for the patient to arrange time for such treatment. Of course, this would not apply to a hospitalized patient where hospital time could be used not only for the treatment of the acute symptoms of hypersensitivity, but also for the purpose of desensitization.

In the group of fifty-eight patients with the hereditary type of hypersensitivity who received coexisting desensitization and hormone therapy, it is impossible to prove, from the data available in this study, that desensitization actually existed, that if desensitization occurred it was solely responsible for improvement, and that ACTH or cortisone was necessary for the accomplishment of desensitization. It is certainly possible to carry out rapid desensitization, without the aid of either of these hormones, in a patient who at the time is not exposed to an offending antigen and who is asymptomatic. We have recently accomplished this in one patient. In this particular instance the same technique of desensitization was employed without accompanying reaction, even though cortisone and ACTH were not administered. However, it is doubtful that one could medicinally treat and desensitize so effectively such patients, who were at the time having marked clinical symptoms, without the aid of cortisone or ACTH. In this group of patients, therefore, it is impossible to specify which factor or factors were responsible for their clinical improvement. The favorable clinical results that were maintained over long periods of time after the withdrawal of cortisone and ACTH lend only suggestive evidence that these hormones did not interfere with the mechanisms of desensitization.

Specific studies were not carried out to determine the presence of blocking antibodies during or after rapid desensitization in the group of fifty-eight patients with the hereditary type of hypersensitivity.

SUMMARY

1. Sixty-six patients, eight with the acquired and fifty-eight with the hereditary type of hypersensitivity, were rapidly desensitized with the aid of ACTH and cortisone.

2. Cortisone or ACTH, administered simultaneously with specific desensitization, does not alter the mechanisms of desensitization.

3. The technique of employing the combination of ACTH or cortisone therapy and rapid desensitization has been successful in the management of

human beings with the acquired type of anaphylactic hypersensitivity who were sensitive to such glandular products as posterior pituitary substances, Pitressin, and insulin, and probably also those with the inherited type of anaphylactic hypersensitivity who were sensitive to pollens, environmentals, and bacterial substances and who, at the time, were having active symptoms. In regard to the latter patients, it is imperative to emphasize clearly that the data so obtained are lamentably difficult to assay; thus, it must be recognized that an accurate estimate of the clinical improvement in the patients with allergic rhinitis, bronchial asthma, and allergic intrinsic eczema is virtually impossible because of the number of clearly variable factors involved.

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NUMBERS AND KINDS OF AIR-BORNE, CULTURABLE FUNGUS SPORES IN HAVANA, CUBA

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INTRODUCTION

IN A previous article[1] we summarized data on the number of culturable fungus spores caught from the air at hourly intervals in Havana. We herewith summarize data on the spore load of culturable fungi in the air at Havana at different seasons throughout the year.

The climate in Cuba is divided into two fairly definite and distinct seasons: (1) the rainy season, from May to November, when torrential downpours are common; and (2) a winter season, from November to May, in the beginning of which gentle rains prevail for a few weeks, followed by dry, relatively cool weather with low relative humidity, which persists until the following rainy season. Seasonal respiratory allergic syndromes reach their greatest vehemence in November; only a few of these patients are sensitive to pollen, and their seasonal allergic disorders are due mostly to molds. It is therefore of considerable importance to accumulate data on the number and kinds of fungus spores in the air at different seasons of the year.

MATERIALS AND METHODS

The collecting apparatus previously described[1] was used in the present studies in order to avoid the influence of the wind, which has a definitive influence upon the apparent quantity of fungus-cultured colonies.

The culture medium used was Sabouraud's agar, acidified to a pH of 5.6 to avoid excessive growth of bacteria. The evidence presented by Swaebly, Christensen, and Grahek[2] indicates that other media may be preferable to Sabouraud's agar in determining the number and kinds of culturable fungus spores in the air. However, any agar medium is likely to be somewhat selective, and Sabouraud's has the merit of having been widely used, so that the results of the present tests should be, in general, comparable with other and similar tests in other places and at other times. Also, since the studies were begun with the use of this medium, it was thought best to continue its use throughout the tests.

Petri dishes were exposed daily, at 10 A.M., and colonies were counted and identified seventy-two hours later.

RESULTS

A total of 5,535 colonies developed on the exposed plates, of which 4,285, or approximately 80 per cent, were identified. The number of colonies of each

Received for publication June 2, 1954.

TABLE I. MOLDS ISOLATED IN THE AIR OF HAVANA

IDENTIFIED MOLDS	ANNUAL TOTAL NUMBER OF COLONIES	AVERAGE PERCENTAGE
Hormodendrum	1,677	30.2
Aspergilli	1,063	19.2
Yeasts	897	16.2
Penicillium	163	2.7
Acrothecium	141	2.5
Mucoraceae	94	1.7
Red mold	62	1.1
Alternaria	49	0.8
Monilia sitophila	24	0.4
Cephalosporium	22	0.3
Orange mold	21	0.3
Chaetomium	20	0.3
Fusarium	15	0.2
Streptomyces	14	0.2
Trichoderma	13	0.2
Helminthosporium	9	0.1
Botrytis	4	0.07
Pullularia	4	0.07
Cephalothecium	4	0.07
Yellow mold	3	0.05
Pink mold	2	0.03
Ambliosporium	1	0.01
Spondylocladium	1	0.01
Botriosporium	1	0.01
Cylindrocephalum	1	0.01
Unidentified molds	1,230	
Total	5,535	

of those identified and the percentage of the total which each comprised are given in Table I.

Hormodendrum (Cladosporium) made up approximately 30 per cent of the total, followed by the Aspergilli with nearly 20 per cent, and yeasts with

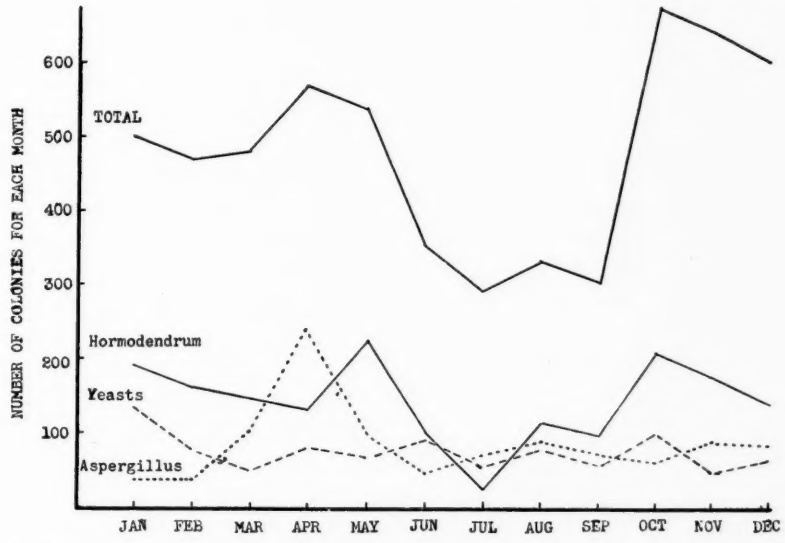

Fig. 1.—Occurrence of atmospheric molds through the year in Havana. Curves based on average total counts for each month, daily Petri dish exposure.

slightly over 16 per cent. Had a medium, such as malt-salt agar, known to be favorable to the growth of Aspergillus species, been used, it is probable that many more colonies of this group would have been detected. Granting the limitations of the techniques used, it is obvious that Hormodendrum (Cladosporium), the Aspergilli, and various yeasts comprised by far the greatest proportion of the microflora detected, with the other twenty-odd genera making up only a small proportion of the total.

As shown in Fig. 1, there were two general peaks of prevalence of mold spores in the air, the first in April and May, at the beginning of summer, and the second in October and November, at the beginning of winter. A study of the seasonal prevalence in 1,200 cases of rhinitis and asthma showed 69 per cent in winter, 7 per cent in summer, and 24 per cent nonseasonal. The data suggest strongly that the periods of high contamination of the air with spores of common molds coincides with times when allergenic symptoms are most severe.

SUMMARY

1. Spores of molds detectable on Sabouraud's agar, exposed at Havana, Cuba, were very abundant from October to February, and in a short period in late May and early April.

2. The principal air-borne molds detected in the present study were Hormodendrum, Aspergillus, and yeasts, with many other genera being encountered occasionally.

3. Of the asthma and rhinitis cases under our observation with sensitivity to fungi, 69 per cent became worse in winter, when the air-borne mold spores were, in our tests, most numerous; only 7 per cent were more pronounced in summer; 24 per cent were severe throughout the year. Thus, the time when the majority of these allergies were most severe coincided with the time when the spores of various fungi known to cause allergenic symptoms were most prevalent in the air of Havana.

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PREVENTION OF BRONCHIAL REACTIONS TO AEROSOLS OF SPECIFIC HAPTENS BY PRETREATMENT WITH AEROSOLS OF PYRIBENZAMINE AND ISUPREL

PRELIMINARY REPORT

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INTRODUCTION

SENSITIZED guinea pigs become dyspneic and may die in anaphylactic shock when exposed to aerosols of specific haptens or antigens. Bronchial desensitization with aerosols of specific haptens in guinea pigs sensitized passively with heterologous antiserum has been studied in this laboratory.[1, 2] The present study was carried out to determine whether prior administration of aerosols of Pyribenzamine and Isuprel would prevent or minimize the typical bronchial reactions to specific hapten aerosols. They were selected arbitrarily as suitable antihistamine and sympathomimetic drugs for these preliminary experiments.

METHOD

Guinea pigs were the experimental animals. Type VI refined antipneumococcal rabbit serum was used as the heterologous passively sensitizing antibody. Passive sensitization was produced by intra-abdominal injections of 0.25 ml. antiserum. Type VI pneumococcal polysaccharide was used as the specific hapten aerosol. Challenging concentrations of the polysaccharide used for the aerosol varied from 1.0 to 3.0 mg. per milliliter. The haptens were nebulized into a glass box large enough to contain three small guinea pigs, without crowding. The aerosol chamber was not quite airtight. The aerosol fog was kept dense enough to ooze visibly from the loose seams. A De Vilbiss No. 40 nebulizer, connected with a source of compressed air bubbled through water, provided the fog.

Positive reactions were graded *slight dyspnea* when the respiratory volume per breath increased definitely and unequivocally, usually with some slowing of respiration. Dyspnea was graded *severe* when it looked as though asphyxia were imminent. Intermediate grades of dyspnea *(slight-moderate, moderate, moderately severe),* were used to describe intermediate degrees of reactions. *A. D.* describes the typical anaphylactic death which occurred in a few animals.

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These experiments were supported in part by Grant #A-283(R) from the National Institutes of Arthritis and Metabolic Disease and by a grant plus supplies of Pyribenzamine by the Ciba Company, Summit, N. J. The antipneumococcal rabbit serum was donated by Dr. B. Scott Fritz of the Wyeth Laboratories, Marietta, Pa. The pneumococcal polysaccharide was donated by Dr. H. D. Piersma of the Lederle Laboratories, Pearl River, N. Y.

Received for publication June 7, 1954.

TABLE I. PREVENTION OF SPECIFIC REACTIONS TO HAPTEN AEROSOLS BY PRETREATMENT WITH AEROSOLS OF PYRIBENZAMINE AND ISUPREL

PYRIBENZAMINE GROUP									
GUINEA PIG	I. A. SENS. DOSE TYPE VI ANTISERUM	CONC. OF PBZ AEROSOL	EXPOSURE TIME (MIN.)	CHALLENGE TYPE VI POLYSAC. AEROSOL	EXPOSURE TIME (MIN.)	REACTION	CHALLENGE TYPE VI POLYSAC. AEROSOL	EXPOSURE TIME (MIN.)	REACTION
	16 to 24 hour interval					16 to 24 hour interval			
1	0.25 ml.	2%	15	1.0 mg. per ml.	10	None	1.0 mg. per ml.	7½	Slight*
2	0.25 ml.	2%	15	1.0 mg. per ml.	10	None	1.0 mg. per ml.	7½	Slight
3	0.25 ml.	2%	15	1.0 mg. per ml.	10	None	1.0 mg. per ml.	9	V. slight*
4	0.25 ml.	1%	10	1.0 mg. per ml.	6	None	1.0 mg. per ml.	2½	Moderate*
5	0.25 ml.	1%	10	1.0 mg. per ml.	6	None	1.0 mg. per ml.	2½	Slight
6	0.25 ml.	1%	10	1.0 mg. per ml.	9	None	1.0 mg. per ml.	8	Slight
7	0.25 ml.	2%	15	1.0 mg. per ml.	8	None	Not done		
8	0.25 ml.	2%	15	1.0 mg. per ml.	5	Sl. mod.*			
9	0.25 ml.	1%	10	1.0 mg. per ml.	6	Slight	3.0 mg. per ml.	2	Moderate
10	0.25 ml.	1%	10	1.0 mg. per ml.	9	Slight	3.0 mg. per ml.	2	Slight
11	0.25 ml.	1%	10	1.0 mg. per ml.	9	Slight	3.0 mg. per ml.	5	Moderate
ISUPREL GROUP									
GUINEA PIG		CONC. OF ISUPREL AEROSOL							
	16 to 24 hour interval					16 to 24 hour interval			
1	0.25 ml.	1:200	15	1.0 mg. per ml.	10	None	1.0 mg. per ml.	9	V. slight
2	0.25 ml.	1:200	15	1.0 mg. per ml.	10	None	1.0 mg. per ml.	5	Slight
3	0.25 ml.	1:200	15	1.0 mg. per ml.	10	None	1.0 mg. per ml.	8	Slight
4	0.25 ml.	1:200	10	1.0 mg. per ml.	12	None	1.0 mg. per ml.	10	Slight
5	0.25 ml.	1:200	10	1.0 mg. per ml.	12	Slight	1.0 mg. per ml.	6	Slight
CONTROL GROUP									
GUINEA PIG									
	16 to 24 hour interval					16 to 24 hour interval			
1	0.25 ml.	0	0	1.0 mg. per ml.	2	Slight	1.0 mg. per ml.	3	Moderate
2	0.25 ml.	0	0	1.0 mg. per ml.	3	Sl. mod.	1.0 mg. per ml.	7½	Slight
3	0.25 ml.	0	0	1.0 mg. per ml.	4	Sl. mod.	1.0 mg. per ml.	3	Moderate

*See text for significance of Slight, V(ery) Slight, Sl(ight) Mod(erate), and Moderate reactions.

EXPERIMENTS

Nineteen guinea pigs were sensitized by the intraabdominal injection of 0.25 ml. Type VI antipneumococcal rabbit serum (Table I). The next day eleven were exposed to Pyribenzamine and five to Isuprel aerosol. Three served as controls.

Five of the Pyribenzamine group were exposed to the aerosol from 2 per cent Pyribenzamine for fifteen minutes. The fog from a 1 per cent concentration of Pyribenzamine was used for ten minutes in the other six. They were then challenged promptly with an aerosol from 1.0 mg. per milliliter of Type VI polysaccharide. The next day nine of the eleven animals were challenged with aerosols of the polysaccharide. The concentration of the polysaccharide used for the specific challenging aerosols was 1.0 mg. per milliliter in three and 3.0 mg. per milliliter in the other six.

Three of the Isuprel group were exposed to the fog from nebulized 1:200 Isuprel for fifteen minutes. The other two were exposed for ten minutes. All five animals were then challenged promptly with the specific polysaccharide aerosol (1.0 mg. per milliliter), three for ten minutes and two for twelve minutes. The next day they were rechallenged with the same concentration of specific aerosol.

The control group of three animals received neither Pyribenzamine nor Isuprel. They were challenged with an aerosol from 1.0 mg. per milliliter of the Type VI specific polysaccharide the day after the sensitizing dose of antiserum. The next day they were rechallenged with the same specific aerosol. It has been shown[1, 2] that nonspecific aerosols do not prevent reactions to specific hapten aerosols.

RESULTS

There were no reactions to the Pyribenzamine aerosol in this experiment, although an occasional pig had dyspnea from it in some preliminary tests. Seven of the eleven pigs did not react to the immediate post-Pyribenzamine polysaccharide aerosol challenge. Three reacted slightly (Nos. 9, 10, 11) and one moderately (No. 8). The next day nine of the Pyribenzamine group were rechallenged. All of them reacted to the specific aerosol. Two (Nos. 9 and 11) of the three which had reacted slightly and slowly to the post-Pyribenzamine challenge reacted more strongly and more quickly the next day. The third one (No. 10) reacted more quickly. The one (No. 8) which had a slight-moderate reaction post-Pyribenzamine and one other pig were not rechallenged the next day, through an oversight.

There were no reactions to the Isuprel aerosol in this experiment. Four of the five did not react to the immediate post-isuprel challenge with specific polysaccharide aerosol. One (No. 5) reacted slightly. All five reacted to the specific polysaccharide challenge the next day. It took twice as long (twelve minutes) for No. 5 to react to the post-Isuprel challenge as it did the next day (six minutes).

The control animals reacted to the initial and to the second specific polysaccharide aerosol challenges.

DISCUSSION

These experiments were done without knowledge of the similar extensive ones by Herxheimer.[3] He used statistical alteration in the "pre-convulsion time" to arrive at conclusions similar to those reported here.

The method described here seems simpler than Herxheimer's[3] in that the chief measure of protection by the drug aerosols is the presence or absence of dyspnea when the sensitized animals are promptly exposed to specific aerosols. Secondary evidences of protection are: (1) the reactions to specific aerosols the next day after the prophylactic effects of the drugs had worn off, and (2) the quicker and/or more intense dyspnea from the specific challenges the next day than immediately after the drug exposures.

More comprehensive studies of the problems suggested by these simple experiments are needed.

CONCLUSIONS

Pretreatment with Pyribenzamine and with Isuprel aerosols prevented or ameliorated reactions to prompt postdrug challenges with specific aerosols in sixteen of seventeen passively sensitized guinea pigs. Reactions were prevented in twelve and ameliorated in four. The protection did not last until the next day. These experiments are preliminary to more detailed studies of the prevention of specific bronchial reactions by the topical use of aerosols of drugs.

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STUDIES IN BACTERIAL ALLERGY*

VII. Bronchial Desensitization and Resensitization in Passively Sensitized Guinea Pigs

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THE experiments presented here are additions to a series of observations[1-3] on different aspects of the broad problem of desensitization with bacterial haptens. The last report[3] was concerned largely with the role of specific hapten aerosols in local desensitization of bronchi. The problem of local resensitization following desensitization is introduced here, together with additional data on local desensitization.

METHODS

Guinea pigs were the experimental animals. Pneumococcal polysaccharides types VI and VIII were used and are referred to as challenging and desensitizing haptens. Types VI and VIII refined antipneumococcal rabbit sera were used as the heterologous, passively sensitizing antibodies.

Passive sensitization was produced by intra-abdominal injections of varying amounts of antiserum.

Challenging and desensitizing concentrations of the polysaccharide haptens varied from 0.1 to 5.0 mg. per milliliter. The haptens were nebulized into a small glass box large enough to contain three small guinea pigs without crowding. The aerosol chamber was not quite airtight. The aerosol fog was kept dense enough to ooze visibly from the loose seams. A DeVilbiss No. 40 nebulizer, connected with a source of compressed air bubbled through water, provided the fog.

Local desensitization was assumed to be present when the guinea pigs were no longer affected by relatively prolonged exposures (eight to ten minutes) to the aerosol fog which previously had caused unequivocal dyspnea.

When testing for local sensitization, the animals were removed from the fog promptly at the onset of unequivocal dyspnea. Failure to do so resulted in three undesirable effects in preliminary experiments: (1) a much higher incidence of anaphylactic death; (2) partial desensitization from the more prolonged hapten exposure; (3) partial desensitization from the progressively more severe reactions which developed when left in the fog.

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Received for publication June 17, 1954.

*This investigation was made possible by Grant No. A-283 (R) from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland, and by donations of pneumococcal polysaccharides by Dr. H. D. Piersma of the Lederle Laboratories, Pearl River, New York, and of antipneumococcal rabbit serum by Dr. B. Scott Fritz of Wyeth and Company's Marietta Laboratories, Marietta, Pennsylvania.

TABLE I. EXPERIMENT I. (A) MULTIPLE PASSIVE SENSITIZATION TO AEROSOLS OF TYPE VI (B) SPECIFIC REACTIONS TO AND DESENSITIZATION WITH TYPE

GUINEA PIG	I.A.* SENS. DOSE ANTISERUM (ML.)			AEROSOL CHALLENGE POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION	AEROSOL DESENS. POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	AEROSOL RECHALLENGE POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	RE-ACTION	CHALLENGE WITH OPPOSITE POLYSAC. (MG./ML.)
	Type VI	*Type VIII*		*Type VI*					*Type VI*			*Type VIII*
1	0.5	0.5		3.0	1	Moderate	0.3	30	3.0	8½	None	3.0
2	0.5	0.5	*16- to 24-Hour Interval*	3.0	1½	Moderate	0.3	30	3.0	8½	None	3.0
3	0.5	0.5		3.0	1	Severe	0.3	30	3.0	8½	None	3.0
4	0.5	0.5		3.0	1	Severe	0.3	30	3.0	9	None	3.0
5	0.5	0.5		3.0	1	A.D.†						
6	0.5	0.5		3.0	1½	A.D.†						
				Type VIII					*Type VIII*			*Type VI*
7	0.5	0.5		3.0	2	Slight to Moderate	0.3	45	3.0	8	None	3.0
8	0.5	0.5		3.0	2	Moderate	0.3	45	3.0	8	None	3.0
9	0.5	0.5		3.0	1½	Moderate	0.3	30	3.0	8	None	3.0
10	0.5	0.5		3.0	1	Moderate	0.3	30	3.0	8	None	3.0

*I.A.—Intra-abdominal.
†A.D.—Anaphylactic death.
‡Animals 9 and 10. No reaction when rechallenged for eight minutes with Type VI (3.0 mg. per milliliter).

Sensitized animals were considered to be locally insensitive when, on first postsensitization exposure, they showed no dyspnea after being in the hapten aerosol for eight to ten minutes.

The animals were observed for several minutes after removal from the fog. The degree of dyspnea recorded in the tables was the maximum observed in this postaerosol period.

Positive reactions were graded *slight dyspnea* when the respiratory volume per breath increased definitely and unequivocally, usually with some slowing of respiration. Dyspnea was graded *severe* when asphyxia seemed imminent. *Slight-moderate, moderate,* and *moderately severe* were used to describe intermediate degrees of dyspnea. *A. D.* describes the typical anaphylactic death which occurred in a few animals. These terms were described in detail previously.[3]

The term *bronchial resensitization* is used when locally desensitized pigs react to subsequent challenges with specific hapten aerosols. Spontaneous resensitization, as used here, means local resensitization which occurs without the use of additional sensitizing antiserum.

Experiment I (Table I).—

Purposes: a. To sensitize passively against two types of pneumococcal polysaccharides by simultaneous intra-abdominal injections of antiserum.

b. To demonstrate the specificity of the hapten reactions by challenging and then desensitizing with one type without preventing reactions and subsequent desensitization to an aerosol of the other type.

OF TYPE VI AND TYPE VIII PNEUMOCOCCAL POLYSACCHARIDES AND TO ACACIA; WITH TYPE VI AND TYPE VIII POLYSACCHARIDES

RE-ACTION	CHALLENGE WITH OPPOSITE POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION	AEROSOL DESENS. POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	AEROSOL RECHALLENGE POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	RE-ACTION	I.A.* SENS. DOSE ANTI-ACACIA SERUM (ML.)		AEROSOL CHALLENGE ACACIA (PER CENT)	EXPOSURE TIME (MIN.)	REACTION
	Type VIII					*Type VIII*							
None	3.0	2	Moderate	0.3	30	3.0	8	None	0.75		3	10	Slight to moderate
None	3.0	8	Slight	0.3	30	3.0	8	None	0.75		3	10	Slight
None	3.0	8	Slight	0.3	30	3.0	8	None	0.75		3	10	Slight
None	3.0	5½	Very slight	0.3	30	3.0	8	None	0.75		3	10	None
	Type VI					*Type VI*				*16- to 24-Hour Interval*			
None	3.0	3	Moderate	0.3	28	3.0	7½	None	0.75		3	10	Very slight
None	3.0	3½	Moderate	0.3	28	3.0	7½	None	0.75		3	10	Slight
None	3.0	1	Moderate to severe	0.3	80	3.0	8	Slight‡	0.75		3	10	None
None	3.0	1	Moderate to severe	0.3	80	3.0	8	Slight‡	0.75		3	10	None

c. To sensitize the same animals passively with antiacacia serum after they had been desensitized with both types of pneumococcal haptens.

This experiment was prompted by previous unsuccessful efforts to produce multiple sensitization.[3] It was assumed that those failures were due to the unfortunate selection of ineffectual antigen-antibody systems.

Preliminary studies showed that antisera given at intervals of two hours intra-abdominally resulted in specific concurrent local sensitization to acacia and to type VI and type VIII pneumococcal polysaccharides. This local sensitization could be demonstrated more regularly to the polysaccharides than to acacia.

Method: Ten guinea pigs were sensitized passively with 0.5 ml. each of type VI and type VIII antiserum intra-abdominally.

Six of these were challenged first with type VI hapten aerosol. The four which survived were desensitized with type VI hapten. They were then challenged and subsequently desensitized with type VIII hapten. The remaining four animals were challenged and then desensitized with type VIII hapten first. They then were challenged and desensitized with type VI hapten.

The same eight survivors were then sensitized passively with antiacacia rabbit serum intra-abdominally. They were challenged the next day with 3 per cent acacia aerosol.

Results: Two of the animals challenged with strong (3 mg. per cubic milliliter) type VI polysaccharide aerosol died promptly in anaphylactic shock.

TABLE II. EXPERIMENT II. DESENSITIZATION WITH INTRA-ABDOMINAL HAPTEN MAY PREVENT REACTIONS TO SPECIFIC HAPTEN AEROSOLS

GUINEA PIG	I.A. SENS. DOSE ANTI-SERUM (ML.)	CONC. OF POLYSAC. USED FOR CHAL-LENGE (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION	I.A. DESENS. WITH POLYSAC. (MG.)			CONC. OF HAPTEN FOR RE-CHALLENGE (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION
					A.M.	NOON	P.M.			
	Type VIII	*Type VIII*			*Type VIII*			*Type VIII*		
		16- to 24-Hour Interval					*16- to 24-Hour Interval*			
1	0.15	1.0	3	Moderate	0.5	1.0	1.5	3.0	8	None
2	0.15	1.0	2	Moderate	0.5	1.0	1.5	3.0	8	None
3	0.15	1.0	2	Moderate	0.5	1.0	1.5	3.0	8	Slight
4	0.5	1.0	8	Moderate	0.5	1.0	1.5	5.0	8	Slight
5	0.5	1.0	8	Moderate	0.5	1.0	1.5	5.0	8	None
6	0.5	1.0	6.5	Moderate	0.5	1.0	1.5	5.0	8	None
	Type VI	*Type VI*			*Type VI*			*Type VI*		
7	0.25	1.0	2	Moderate	0.5	1.5	2.5	3.0	8	None
8	0.25	1.0	2	Severe	0.5	1.5	2.5	3.0	8	None
9	0.25	1.0	3	Moderate	0.5	1.5	2.5	3.0	8	None
10	0.25	1.0	1	Severe	0.5	1.5	2.5	3.0	8	None.

The four which were challenged and then desensitized first with type VI hapten aerosol reacted later when challenged with the type VIII aerosol and were subsequently desensitized specifically by it.

The four animals challenged and desensitized first with type VIII subsequently reacted to and were desensitized by type VI hapten aerosol.

Four of the eight animals sensitized with antiacacia serum reacted definitely to the aerosol challenge. Desensitization of these was not attempted, since it had been accomplished many times previously in acacia-sensitive animals. The reaction of another was, perhaps, equivocal. The other three showed no reaction. This percentage of reactions to acacia aerosol is comparable to that of other animals sensitized with the same batch of antiacacia rabbit serum.

Conclusions: 1. Specific local passive sensitization to multiple aerosol haptens and antigens can be conferred by suitable antisera.

2. Local desensitization may be produced by aerosols specifically, one at a time.

Experiment II (Table II).—

Purpose: To see if intra-abdominal hapten injections will desensitize locally against aerosols of haptens.

Method: Three groups of animals were used. The first three animals were sensitized with 0.15 c.c. of type VIII antiserum. They were challenged with type VIII hapten aerosol (1.0 mg. per milliliter) to demonstrate sensitization. They were then given three desensitizing injections of 0.5, 1.0, and 1.5 mg. of type VIII polysaccharide intra-abdominally in one day.

The day after desensitization they were challenged with three times (3.0 mg. per milliliter) the initial reacting concentration of hapten aerosol.

The second group of three animals was sensitized with an arbitrarily larger dose, 0.5 c.c., of type VIII antiserum. They were challenged with type VIII aerosol hapten (1.0 mg. per milliliter) to demonstrate sensitization. They were desensitized by the same method and dose as the first group. They were challenged the day after desensitization with five times (5.0 mg. per milliliter) the initial reacting concentration of polysaccharide aerosol.

The third group of four animals was sensitized with 0.25 ml. of type VI antiserum. They reacted to the initial challenge with 1.0 mg. per milliliter hapten aerosol.

They were desensitized with three larger doses of polysaccharide intra-abdominally, 0.5, 1.5, and 2.5 mg. in one day. The next day they were challenged with three times the initial reacting concentration of hapten aerosol.

Results: Two of three animals in both of the first two (type VIII) groups and all four of those in the third (type VI) group were completely desensitized locally. The intensity of the reaction was significantly reduced in the two animals (Nos. 3 and 4) which were not completely desensitized.

In eight other animals (not in the table) in which the initial reaction to aerosol challenge was slight, desensitization by the intra-abdominal polysaccharide was complete.

Conclusions: Desensitization with haptens given intra-abdominally may prevent reactions to specific hapten aerosols. Larger doses of hapten intra-abdominally probably would have provided complete protection in pigs 3 and 4.

Experiment III (Table III).—

TABLE III. EXPERIMENT III. FAILURE TO ELICIT REVERSE PASSIVE SENSITIZATION REACTIONS WITH AEROSOL ANTISERUM BUT ANAPHYLAXIS FOLLOWED INTRAVENOUS ANTISERUM

GUINEA PIG	I. V. SENS. DOSE TYPE VI POLYSAC. (MG.)		AEROSOL TYPE VI 1:5 ANTISERUM EXPOSURE TIME (MIN.)	REACTION	I. V. CHALLENGE TYPE VI CONC. ANTISERUM (ML.)	REACTION
1	2.0		10	None	2.0	Severe
2	2.0		10	None	2.0	Severe
3	2.0		10	None	2.0	A. D.
4	2.0	*16- to 24-Hour Interval*	10	None	2.0	Severe
5	2.0		10	None	2.0	A. D.
6	1.0		8	None	2.0	Moderate
7	1.0		8	None	2.0	Moderate
8	1.0		8	None	1.0	Moderate
9	1.0		8	None	1.0	Moderate

Purpose: To attempt to produce reversed passive allergic bronchial reactions with an intravenous hapten as the sensitizer and specific antipneumococcal rabbit serum as the aerosol antibody.

Method: Nine pigs were sensitized intravenously with 1.0 or 2.0 mg. of type VI pneumococcal polysaccharide. They were challenged one hour later with an aerosol of type VI antipneumococcal rabbit serum. After a negative aerosol challenge, they were given antipneumococcal type VI antiserum intravenously to test for systemic reversed passive sensitization.

Results: There were no reactions to the antiserum aerosol. All the animals had anaphylactic shock when challenged intravenously with the antiserum.

Two of three animals similarly treated with type VIII polysaccharide and antiserum had equivocal reactions to the antiserum aerosol.

A number of other animals similarly treated, with other antigen-antibody systems, failed to react to the antiserum fog.

Conclusions: Definite reversed, passive, anaphylactic reactions to nebulized antiserum were not produced, although anaphylaxis followed intravenous injections of the same antiserum.

Experiment IV (Table IV).—

Purposes: To resensitize bronchi passively after they have been desensitized locally.

Method: Preliminary experiments showed that guinea pigs could be sensitized with 0.05, 0.1, and 0.15 ml. of antipneumococcal rabbit serum intra-abdominally.

Six guinea pigs (1 to 6 in table) were sensitized, three with type VI and three with type VIII antiserum. Sensitization was confirmed by positive reactions to a relatively weak concentration (1.0 mg. per milliliter) of the hapten aerosol. They were then desensitized by an aerosol of one-tenth (0.1 mg.

TABLE IV. EXPERIMENT IV. (A) RESENSITIZATION OF DESENSITIZED BRONCHI BY REPEATING THE INTRA-ABDOMINAL SENSITIZING DOSE OF ANTISERUM. (B) BRONCHI NOT SENSITIZED BY SMALL DOSES OF ANTISERUM MAY BECOME SENSITIZED BY ADDITIONAL DOSES. (C) LOCAL DESENSITIZATION MAY BE EFFECTIVE AGAINST THIRTY TIMES THE DESENSITIZING CONCENTRATION OF HAPTEN AEROSOL

GUINEA PIG	I. A. SENS. DOSE ANTISERUM (ML.)		AEROSOL CHALLENGE POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION	RECOVERY TIME (MIN.)	AEROSOL DESENS. POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	AEROSOL RECHALLENGE POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION	I. A. RESENS. DOSE ANTISERUM (ML.)		AEROSOL CHALLENGE POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION
	Type VI		*Type VI*				*Type VI*		*Type VI*			*Type VI*		*Type VI*		
1	0.05		1.0	1½	Moderate	27	0.1	45	3.0	8	None	0.05		1.0	9½	Slight
2	0.15		1.0	4½	Slight	8	0.1	45	3.0	8	None	0.15		1.0	2	Slight
3	0.25		1.0	1	Slight to moderate	28	0.1	45	3.0	8	None	0.25		1.0	7	Moderate
	Type VIII		*Type VIII*				*Type VIII*		*Type VIII*			*Type VIII*		*Type VIII*		
4	0.05		1.0	3	Slight to moderate	12	0.1	45	3.0	8	None	0.05		1.0	9	None
5	0.15		1.0	5	Slight	5	0.1	45	3.0	8	None	0.15		1.0	6	Slight
6	0.25		1.0	5	Slight to moderate	15	0.1	45	3.0	8	None	0.25		1.0	6	Slight
	Type VI		*Type VI*									*Type VI*		*Type VI*		
7	0.05		3.0	8	None							0.1		3.0	9	Slight
8	0.05		3.0	8	Very slight	4						0.1		3.0	9	Slight

16- to 24-Hour Interval (spanning both groups)

TABLE V. EXPERIMENT V. LARGE DOSES OF SENSITIZING ANTISERUM MAY PRODUCE SPONTANEOUS RESENSITIZATION (A) SEVEN AND (B) TWO DAYS AFTER LOCAL DESENSITIZATION BY SPECIFIC HAPTEN AEROSOL

GUINEA PIG	I. A. SENS. DOSE TYPE VI ANTISERUM (ML.)		CHALLENGE TYPE VI POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION	DESENS TYPE VI POLYSAC. AEROSOL (MG./ML.)	DESENS TYPE VI POLYSAC. AEROSOL TIME IN MIN.		CHALLENGE TO CONFIRM DESENS. CONC. TYPE VI POLYSAC. AEROSOL (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION		CHALLENGE TYPE VI POLYSAC. (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION
								*A**							
1	5.0	*16- to 24-Hour Interval*	1.0	½	Moderate	1.0	84	*1- to 2-Hour Interval*	3.0	8	None	*7-Day Interval*	3.0	7½	Sl. mod.
2	5.0		1.0	1	Severe	1.0	84		3.0	8	None		3.0	4	Moderate
3	5.0		2.0	½	Moderate	0.5	120		3.0	8	None		2.0	8	Sl. mod.
4	5.0		2.0	½	Mod. Sev.	0.5	120		3.0	8	None		2.0	8	None§
								Controls							
5	5.0					NINE-DAY INTERVAL							3.0	2	Severe
6	5.0												3.0	2	Moderate
								B†							
7	5.0	*16- to 24-Hour Interval*	2.0	1	Mod. Sev.	0.5	60	*1- to 2-Hour Interval*	3.0	8	None	*2-Day Interval*	3.0	5	Mod. sev.
8	5.0		2.0	2	Severe	0.5	60		3.0	8	None		3.0	8	Slight
9	5.0		2.0	1	Mod. Sev.	0.5	60		3.0	8	None		3.0	7½	Moderate
10	5.0		2.0	8	Slight	0.5	60		3.0	8	None		3.0	7½	Slight
11	5.0		2.0	½	Moderate	0.5	120		3.0	8	None		2.0	8	None§
12	5.0		2.0	½	Severe	0.5	120		3.0	8	None		2.0	8	None§
13	5.0		2.0	½	A. D.										
								Controls‡							
14	None		2.0	10	None	0.5	60	*1- to 2-Hour Interval*	3.0	8	None	*2-Day Interval*	3.0	8	None
15	None		2.0	10	None	0.5	60		3.0	8	None		3.0	8	None
16	None		2.0	10	None	0.5	60		3.0	8	None		3.0	8	None
17	None		2.0	10	None	0.5	60		3.0	8	None		3.0	8	None

*No effort was made to desensitize any of these pigs after initial desensitization.

†Pigs 7 to 12 were rechallenged on the fourth, fifth, and sixth days; Nos. 7 to 10 reacted to each challenge while Nos. 11 and 12 did not react to either rechallenge.

‡Pigs 14 to 17 were negative controls. They, too, were exposed to 3.0 mg. per milliliter of polysaccharide on the fourth, fifth, and sixth days. They did not react.

§Failure to become resensitized spontaneously.

per milliliter) of the reacting concentration of the hapten. Desensitization was confirmed by a negative aerosol challenge with thirty times (3.0 mg. per milliliter) the desensitizing concentration of hapten. After desensitization the initial intra-abdominal sensitizing dose of antiserum was repeated.

Two other pigs (7 and 8) were given initial sensitizing doses of 0.05 ml. of antiserum. They had no or equivocal reactions to the first (strong, 3.0 mg. per milliliter) aerosol hapten challenge. They were then given an additional 0.1 c.c. of antiserum intra-abdominally. They were rechallenged the next day with the strong aerosol concentration which initially had failed to elicit significant reactions.

Results: None of the pigs reacted to the desensitizing concentration (0.1 mg. per milliliter) of the hapten. Five of the six animals which had been desensitized locally by the weak hapten aerosol were resensitized by repeating the initial sensitizing dose of antiserum. The one which was not resensitized was one of the two which received the smallest resensitizing dose of antiserum. The other two, which had been sensitized very slightly or not at all by the first small dose of antiserum, became sensitized when additional sensitizing doses of antiserum were given.

Conclusions: 1. Locally desensitized guinea pigs can be resensitized usually by repeating the initial intra-abdominal dose of sensitizing antiserum.

2. Guinea pigs which do not become sensitized locally by small intra-abdominal doses of antiserum may become sensitive if subsequent larger sensitizing doses are given.

3. Local desensitization may be effected by subreacting concentrations of the hapten aerosol. This desensitization may be effective against thirty multiples of the desensitizing concentration of the hapten.

Experiment V (Table V).—

Purpose: To determine whether spontaneous local resensitization will occur after local desensitization in guinea pigs previously sensitized with very large doses of antiserum.

In earlier experiments,[3] using small sensitizing doses of 0.65 ml. of antiserum or less, local desensitization persisted nine to eleven days (about as long as passive sensitization lasted) and spontaneous resensitization was not observed.

Method and results: Thirteen guinea pigs were sensitized with 5.0 ml. type VI antiserum. The next day eleven of them reacted when challenged with type VI pneumococcal polysaccharide aerosol. The ten survivors were desensitized promptly with 1.0 or 0.5 mg. per milliliter hapten aerosol. Desensitization was confirmed by failure to react when challenged with a stronger (3.0 mg. per milliliter) aerosol. Seven days (Nos. 1 to 4) and two days (Nos. 7 to 12) later they were rechallenged with type VI polysaccharide aerosol. Seven of the ten pigs reacted.

Two pigs (Nos. 5 and 6) were not desensitized. They were used as positive controls and were challenged with type VI polysaccharide on the ninth day. They reacted promptly.

Four pigs (Nos. 14 to 17) were not sensitized. They were negative controls. They did not react to any of the aerosol challenges.

Conclusions: 1. Guinea pigs passively sensitized with large amounts of antiserum may remain sensitized for at least nine days.

2. Seven of ten such animals had become spontaneously resensitized locally when challenged seven or two days after effective local desensitization by an aerosol of specific hapten. Three did not become resensitized.

3. This is in contrast to the persistence of local desensitization for at least nine days when small doses of sensitizing antiserum were used.[3]

4. Some unrecognized factor other than the administration of ±30 multiples of a normally sensitizing dose of antiserum is necessary to produce spontaneous resensitization at will.

DISCUSSION

It was stated earlier[3] that: "It would have emphasized the specificity of the desensitization if some guinea pigs had been sensitized to antigens or haptens and had then been desensitized, specifically against one, without prevention of the aerosol reaction to the other." Experiment I provides this emphasis of the specificity of the aerosol desensitization. In addition, it shows that guinea pigs may be passively sensitized so they will react locally and specifically to aerosols of three, and presumably more, haptens or antigens. The earlier unsuccessful attempts[3] are not completely explained. Partial explanations lie in: (1) the unfortunate selection of the type II pneumococcal antibody-polysaccharide system, which would not sensitize;[3] (2) the fact that passive local sensitization of the bronchi is much less consistently demonstrated by aerosols, both in degree and incidence, than is systemic sensitization by intravenous challenge. This is true of the six antigen-antibody systems used so far in this laboratory.

It has been shown[3] that aerosol desensitization is local, that is, subsequent intravenous haptens will cause prompt anaphylaxis. Experiment II shows that the converse of this is not true. In other words, the bronchi of guinea pigs desensitized by intra-abdominal injections of the hapten did not react to the postdesensitization aerosol hapten challenge. It is assumed that these pigs were systemically desensitized, since earlier experiments[2] had shown that smaller doses of hapten would protect against intravenous challenge with the polysaccharide.

A ready explanation of the broader effectiveness of intra-abdominal versus aerosol desensitization seems to lie in the great differences in the amounts of hapten used in the two procedures. Intra-abdominal desensitization required 3 to 4.5 mg. of the hapten, whereas in local desensitization by aerosol only about 350 gamma of hapten is inhaled per hour (using Feinberg's[4] estimates of antigen inhaled by aerosol). No method has been devised yet for estimating how much inhaled antigen gets into the circulation.

The importance of the quantity of hapten used in intra-abdominal desensitization against local reactions to aerosols is suggested by these observa-

tions: (1) All four animals receiving the larger desensitizing doses, in Experiment II, were completely protected. (2) The smaller desensitizing doses protected only two of three animals in each of the first two groups.

It is of interest that all eight of the animals, mentioned but not tabulated in Experiment II, which reacted only slightly to the initial aerosol challenge were completely desensitized by the smaller intra-abdominal doses of hapten.

No effort has been made to explain the unsuccessful attempts to elicit dyspnea in the reverse passive aerosol anaphylaxis experiment (III). It is probable that too little antiserum is absorbed by aerosol[4] to mediate a reaction. It is not likely that massive sensitizing doses of hapten would be successful.

Passing attention should be given to the observation in Experiment IV that very weak nonreacting concentrations of hapten aerosol desensitized completely against thirty multiples of the desensitizing concentration. This is more impressive than the fifteen to twenty multiples previously reported.[3]

In the preceding article[3] it was shown that local desensitization lasted nine to eleven days, under the conditions of those experiments. Herxheimer[5] reported that actively sensitized guinea pigs desensitized by an aerosol of a whole antigen became resensitized spontaneously in a few days. It seemed proper to attempt to reconcile these two seemingly contradictory conclusions. Examination of the earlier data[3] suggested that the observed failure to become resensitized might be due to amounts of circulating antibody insufficient to induce spontaneous local resensitization after local desensitization. Experiments IV and V were designed to test this hypothesis.

The results of Experiment IV supported this hypothesis in three ways: (1) animals which were not sensitized by the initial small sensitizing dose became sensitive when additional sensitizing antiserum was given; (2) most of the locally desensitized animals were resensitized when the original sensitizing dose was repeated; (3) the desensitized animal which did not become resensitized was one of two which received the smallest sensitizing and resensitizing doses of antiserum.

The results in Experiment V give additional support to the hypothesis. In the earlier experiments,[3] in which local desensitization was lasting, the sensitizing doses of antiserum were small, roughly, 3.5 to 4.5 multiples of the nearly minimal effective sensitizing dose of 0.15 c.c. used in Experiment IV. When 33.3 multiples (5.0 c.c.) of this small (0.15 c.c.) sensitizing dose were used in Experiment V, spontaneous resensitization occurred. It seems reasonable to assume that the large (5.0 ml.) sensitizing dose of antiserum supplied enough excess circulating antibody (a) to neutralize the small amount of desensitizing hapten locally and subsequently (b) to resensitize the bronchi.

Antibody production in actively sensitized guinea pigs continues long enough to maintain active sensitization for several months.[6, 7] The assumption seems reasonable, therefore, that there was enough excess antibody to explain the spontaneous resensitization in Herxheimer's[5] actively sensitized animals. It should take very little to replace the antibody neutralized by the ±350 gamma per hour of haptens inhaled in desensitization.

The problem of spontaneous resensitization is not as simple as the preceding discussion would lead one to believe. Other unrecognized factors are undoubtedly concerned. This opinion is derived from two attempts to titrate, roughly, the smallest amounts of type VIII and type VI antisera which would produce spontaneous resensitization. The results with type VIII were so at variance with those with type VI antiserum that further study is needed to resolve the differences.

Spontaneous resensitization has received scant attention in the recent literature. It is obviously important in any consideration of the duration of desensitization. It seems likely that a quantitative relationship between sensitizing antibody and desensitizing hapten or antigen will be shown to be the crux of the problem. Such a relationship already has been reported in reverse passive anaphylaxis resensitization.[8]

No hypothesis is offered to explain the marked variations observed in: (1) the incidence, intensity, and time of onset of the reactions to specific aerosols; (2) the desensitizing time; (3) the concentration of effective challenging doses; (4) the amounts of sensitizing antiserum necessary to produce spontaneous resensitization; and (5) the efficiency of different antigen-antibody systems. This is in striking contrast to the relatively uniform reactions to intravenous challenges obtained in similarly sensitized animals.

SUMMARY AND CONCLUSIONS

These additional studies of bacterial haptens in local desensitization of passively sensitized guinea pigs permit the following conclusions:

1. Suitable antisera can confer multiple passive sensitization to guinea pigs so they will react to and be desensitized specifically by aerosols of each hapten, one at a time.

2. Specific bronchial desensitization may be induced by intra-abdominal injections, as well as by the previously reported aerosols of haptens.

3. Attempts to elicit reverse passive anaphylactic reactions to nebulized antiserum were unsuccessful, although anaphylaxis followed intravenous injections of the same antiserum.

4. Locally desensitized bronchi can be resensitized, usually, by repeating the intra-abdominal dose of sensitizing antiserum.

5. Subsequent larger intra-abdominal doses of antiserum may sensitize when earlier smaller doses do not.

6. Local desensitization of bronchi may be effected by subreacting concentrations of hapten aerosol. This desensitization may be effective against thirty multiples of the desensitizing concentration of hapten.

7. Spontaneous local resensitization was observed two days and seven days after effective local desensitization in some, but not all, animals sensitized passively with large amounts of antiserum.

8. The problem of resensitization has received scant attention in the past. It needs more study.

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ACTION OF HISTAMINE LIBERATOR COMPOUND 48/80 IN THE GUINEA PIG*

With a Review of Previous Reports of Its Action in Other Species

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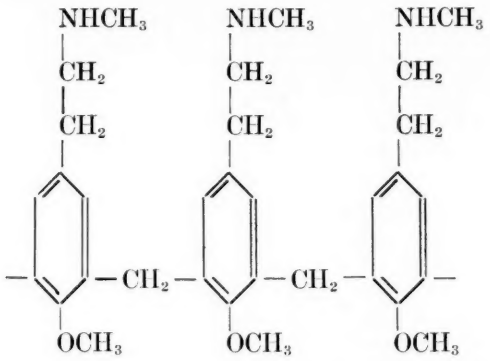
HISTAMINE may be released from tissues by a number of agents. Among these one of the most recently described and most potent substances is compound 48/80, a condensation product of p-methoxyphenylethyl methylamine with formaldehyde. This compound was prepared first by Baltzly, Buck, de Beer, and Webb[1] and is considered to be a mixture of dimers, trimers, and tetramers, with a structure of the following general type:



As with other histamine liberators,[2] the release of histamine by compound 48/80 is followed by a relative and temporary depletion of histamine, varying in degree and duration with the technique used and the species of animal employed. While many of the other histamine liberators are active in only one or two species of animals which differ with each substance, compound 48/80 is effective in most species, including man.

Since the guinea pig is the laboratory animal resembling man most closely in degree of histamine sensitivity and in the shock organs commonly involved in hypersensitiveness it was felt that this animal might constitute a suitable type for investigations with compound 48/80.

Previous reports on the action of this compound concern several other species of animals. The action of this compound has also been observed to

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Received for publication Aug. 18, 1954.

*This investigation was supported in part by Research Grant No. G-3848 from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

some extent in the guinea pig. It was considered pertinent, therefore, to review the entire subject of compound 48/80, to serve as a basis of comparison with the results of our experiments.

PREVIOUS INVESTIGATIONS WITH COMPOUND 48/80

Compound 48/80 has been claimed to be an effective histamine liberator in the cat, dog, rat, mouse, and man, but rather ineffective in the rabbit.

In the cat, Paton[3] and Dews and his associates[4] found a marked fall in blood pressure after a small intravenous dose. The plasma histamine was increased about twenty fold.[3] In a perfused skin preparation an intra-arterial injection produced a marked release of histamine. Feldberg and Paton[5] demonstrated a similar release from an isolated perfused gastrocnemius muscle preparation. Smith[6] found that the histamine content of the cat's skin normally showed regional differences similar to those in other species. An intravenous injection of compound 48/80 reduced the histamine in the skin. Intraperitoneal injection of large doses resulted in prostration, vascular collapse, respiratory distress, and gastrointestinal disturbances. With recovery there was erythema and facial edema. These symptoms decreased with repeated injections and there resulted an 80 per cent depletion of the histamine in the skin. In animals treated with repeated injections of compound 48/80 or with large doses of histamine in beeswax, an intravenous infusion of histamine produced a much smaller rise in blood histamine than in untreated animals. Smith[7] found also that an intravenous injection of 5γ per kilogram or more of compound 48/80 produced acid gastric secretion. His experiments indicated that this was due to release of histamine from distant organs and not from the stomach.

Investigations on the effect of compound 48/80 in dogs produced results much like those in the cat. Blood pressure fall was noted by Paton,[3] as well as by Dews and associates.[4] The latter group found that recovery of pressure level took two hours or more and that, orally, large amounts of this substance were nonlethal. Paton found an increase in blood coagulation time. After the plasma histamine had returned to normal a second dose of the compound failed to increase the histamine level or the clotting time. Feldberg and Schachter[8] injected the compound into the isolated skin flap of the dog with resulting histamine release and marked edema. Subsequent injections produced no further histamine release. Subcutaneous repeated administration produced a state of refractoriness to the drug, lasting two to seven days, as noted by Paton and Schachter.[9] Feldberg and Talesnik[10] injected 500γ of compound 48/80 into the saphenous artery. At intervals of several hours to days, excised skin areas from the leg were assayed for histamine. There was marked histamine depletion up to 85 per cent of the total available, and recovery of histamine was not complete for fifteen or more days.

Intraperitoneal injection of 500γ of this compound in rats was found by Feldberg and Talesnik[10] to produce itching, cyanosis, prostration, and frequent deaths. A dose of 100 to 200γ resulted in marked swelling of the paws

and face, but there were no fatalities. Subsequent daily injections of the same dose produced less effect, and finally higher doses, even those exceeding previously fatal ones, could be used. Histamine depletion, of about 90 per cent, occurred in the skin and cardiac skeletal muscle, but very little depletion resulted in the liver, stomach, or duodenum. Rats having prolonged intraperitoneal treatment with compound 48/80 failed to show edema after an injection of 2 ml. egg white. Protection was also present in animals made photosensitive with hematoporphyrin. Shaved areas, 2 by 1 cm., on the abdomen of the rat were injected subcutaneously with 500γ of compound 48/80 in 1 ml. normal saline. At varying intervals such skin areas were removed, ground, and assayed for histamine. About 90 per cent depletion occurred. The histamine gradually returned, but was still incomplete after forty days. The subcutaneous injection did not affect the remainder of the skin. The symptoms and toxicity of compound 48/80 in mice were about the same as those in rats. It has been noted also by Dews and his associates[4] that large oral doses of this compound in mice do not produce fatalities.

Mongar[11] employed minced guinea pig lung to test the histamine-releasing potential of a variety of a homologous series of amines, C_nH_{2n+1} NH_2 HC_1 up to C_{18}. The C_{10} compound was highly active, seven times more active than compound 48/80. However, when tested in the human skin the latter was 1,000 times more active than the C_{10} compound. The question raised by him is whether the wheal test is a true measure of histamine-releasing activity. The discrepancy led to the question of whether the activity of histamine releasers varies when compared by different methods. Feldberg and Mongar[12] compared the extent of histamine released by compound 48/80 and octylamine from isolated perfused tissues of several species of animals. On the skin flaps and gastrocnemius muscle of the cat, 48/80 was 200 times more active than octylamine. On the perfused hindquarters of the rat compound 48/80 was more than 100 times as active as octylamine, while in the same tissue of the guinea pig it was sixty times more active. In release of histamine in the perfused lung, the ratio of the activity of compound 48/80 compared to octylamine was 20:1 in the cat, 2 to 4:1 in the rat, and 1:1 in the guinea pig.

Mongar and Schild[13] compared the histamine-releasing effects of egg albumin with those of compound 48/80 on thin pieces of various tissues of guinea pigs sensitized to egg albumin. It was noted that 1 mg. per milliliter of compound 48/80 gave the same effect as a similar concentration of egg albumin. The percentage of available histamine released by the antigen or the chemical releaser varied considerably with different tissues, being least in the intestine (9 per cent) and most in the diaphragm (43 per cent), aorta, and uterus. The skin and lung yielded only 10 per cent of their histamine. The percentage of histamine released was not related to the total amounts in the tissue. However, the largest amounts released were from organs richest in histamine (lungs, aorta, and uterus). In the sensitized lung and aorta, compound 48/80 depleted histamine, and the antigen did not release additional amounts. However, compound 48/80 produced further histamine release after an initial release by the antigen. In the small intestine, compound 48/80

caused marked potentiation of antigen release of histamine. In the uterus, the depletion of histamine by compound 48/80 resulted in very little histamine release by further action of the antigen, in spite of the fact that the antigen caused contraction. This finding can be cited in support of a nonhistamine mediation of antigen-antibody effect.

The acute toxicity from compound 48/80 was studied by Dews and his associates[4] in guinea pigs, rats, and mice. In mice, LD_{50} intravenously was 3.1 mg. per kilogram, the major symptoms consisting of tremors, muscular weakness, slowed respiration, and occasional agonal convulsion. In rats, the LD_{50} dose was around 5 mg. per kilogram with symptoms of prostration, hyperemia and cyanosis of the extremities, and edema. The symptoms in the guinea pig resembled those of rats, while some showed respiratory embarrassment. Feldberg and Miles[14] injected compound 48/80 (2.5 to 5.0 mg. per kilogram) intravenously in guinea pigs twenty minutes after an intravenous injection of Pontamine Sky Blue (1.3 ml. per kilogram of 5 per cent). The different areas were blued in proportion to their histamine content. Miles and Miles,[15] using Pontamine Sky Blue, were able to show intensified bluing in the areas injected with compound 48/80 or histamine. When Dews and his associates[4] exposed guinea pigs to an aerosol of a 2 to 5 per cent solution of compound 48/80 they noted that "asphyxial collapse was sometimes observed."

Ungar and his associates[16] incubated guinea pig lung suspension with compound 48/80, among other agents, and examined the suspending fluid at intervals. They claim that the results indicate that in anaphylactic and anaphylactoid reactions the following sequence of events takes place: profibrinolysin activation, appearance of active fibrinolysin, breakdown of proteins to which histamine is bound, release of free histamine.

The rabbit appears to have a response different from other animals. In perfusion experiments in sensitized rabbits[17] the ear released large amounts of histamine with compound 48/80 or with antigen, although more by the latter. The liver and intestine, however, failed to liberate histamine when perfused with compound 48/80, although the antigen did liberate it. The antigen given intravenously increased the plasma histamine two to several fold, but compound 48/80 produced no increase in histamine. Rabbits injected with Pontamine Sky Blue showed no increased bluing after an injection of compound 48/80. However, the injection of the specific antigen produced marked localized bluing, particularly in the skin, gastrointestinal tract, and lungs. These findings are substantiated by those of Dews and his collaborators who obtained no fall in blood pressure from moderate amounts of compound 48/80. Large amounts produced a rise in pressure.

Reports on investigations with this compound in man are meager. Paton[3] reported that wheals and flares were produced in the human skin by intradermal injection. Ehrlich, Rhines, and Samter[18] observed that the skin response was proportional to that obtained with histamine in the same individual. Our laboratory[2] confirmed these observations and showed that the degree of reactivity of the human skin to this compound as compared to histamine (base) on the basis of actual weight was about 1:3 to 1:9. Skin sites

were moderately depleted of histamine for two to six days. We showed further that a specific antigen could also make the site refractory to a heterologous antigen and to compound 48/80.

Mota and his associates[19] have demonstrated rupture of the mast cells of the rat in vivo and in vitro by compound 48/80. Norton used this finding[20] for the basis of an assay which is now being employed by de Beer and associates to determine the potency of new batches of compound 48/80. Dews and his associates[4] showed that this compound produced no toxic effect on the heart of the rat, guinea pig, or dog. The same investigators noted that Neo-antergan had only a moderate inhibiting effect on the edema in rats produced by compound 48/80.

PROCEDURE AND RESULTS

1. *Effect of Intraperitoneal Injections of Compound 48/80 on Reactivity to the Same Compound.*—Guinea pigs were given intraperitoneal injections of compound 48/80,* as either a single sublethal dose, a series of daily sublethal doses, or a series of daily doses beginning with small amounts and increasing to the maximum tolerated dose. Three different batches of the compound were used and, since their toxicities differed, it was necessary to use the same compound for each individual experiment and its corresponding control group. The order of increasing potency of the compound was as follows: 48/80a, 48/80d,[2] and 48/80d. Fifteen control guinea pigs given intraperitoneal injections of 3 mg. per kilogram of "a" had severe symptoms, consisting of itching, sneezing, retching, and prostration. Dyspnea was not an obvious feature. At 4 mg. per kilogram, some animals died, and at higher doses fatality was the rule. A mixture of equal parts of "a" and "d" gave the following mortality rates: 3 mg. per kilogram, 12/18; 10 mg. per kilogram, 12/12. Compound "d[2]" produced no deaths in doses of 2 mg. per kilogram. The gross findings on autopsy were markedly congested and small lungs and a congested liver.

(a) Single dose: Twelve guinea pigs injected intraperitoneally with compound "a," 3 mg. per kilogram (control mortality 0/15), were divided into two groups of six each. Twenty-four hours later the animals were reinjected with the same compound. Group 1 received 4 mg. per kilogram, of which one died (control mortality 2/10). The other group received 5 mg. per kilogram and four animals died (control mortality 7/10). A mixture of equal quantities of "a" and "d" was given in a dose of 3 mg. per kilogram to another series of eighteen animals, twelve of which died. Five of the survivors were given a similar dose three days later and two of these animals died.

Six animals were given 3 mg. per kilogram, intraperitoneally, of compound 48/80a. The following day they were exposed to an aerosol of the compound from a solution 20 mg. per milliliter. The animals did not show typical asthmatic breathing,[21] but the end point was marked scratching or some respiratory embarrassment. The lungs of such animals did not show the

*Kindly furnished by Dr. E. J. de Beer, Wellcome Research Laboratories, Tuckahoe, New York.

emphysema seen with inhalation of histamine or an antigen aerosol. The average time required to produce symptoms was seven minutes and fifteen seconds. A control group of six animals, which was not pretreated with an intraperitoneal injection, had average reacting time of seven minutes and fifty seconds. In another experiment with a different batch of the compound the time between the intraperitoneal injection and the aerosol was shortened to one hour. Twelve animals receiving the aerosol one hour after the injection had an average reaction time of three minutes and thirty-five seconds. The animals receiving no previous intraperitoneal injection had an average reacting time of five minutes and five seconds. Six of the animals receiving both the intraperitoneal and aerosol doses, whose average reaction time to the aerosol was three minutes and seventeen seconds, were kept in the chamber as long as they could tolerate the aerosol without serious symptoms, but not longer than ten minutes. One hour later they were re-exposed to the aerosol, with an average reaction time of five minutes and twenty seconds. The other six experimental animals, whose average reaction time to the aerosol was four minutes and two seconds, were re-exposed to the aerosol three hours later, with an average reacting time of four minutes and thirty-five seconds.

(b) Gradually increasing doses: Six guinea pigs were given intraperitoneal injections of 48/80 at intervals of one day or longer as follows: Nov. 16, 1953, 50γ/kg.; Nov. 17, 1953, 100γ/kg.; Nov. 18, 1953, 200γ/kg.; Nov. 19, 1953, 800γ/kg.; Nov. 20, 1953, 1.6 mg./kg.; Nov. 23, 1953, 1.6 mg./kg.; Nov. 24, 1953, 3 mg./kg.; Nov. 25, 1953, 3 mg./kg.; Nov. 27, 1953, 3 mg./kg.; Nov. 30, 1953, 2 mg./kg.; Dec. 1, 1953, 3 mg./kg.; Dec. 2, 1953, 3 mg./kg. From the very beginning of the injections the animals showed symptoms, first consisting of itching. As the doses progressed the itching became more marked, and retching, muscle weakness, and ruffled skin were present in some. On November 25 one of the animals, which had shown prostration on the preceding day from a dose of 3 mg. per kilogram, died after convulsions from a similar dose. On November 27 there was a death in an animal receiving 3 mg. per kilogram, which suffered only itching two days before from a similar dose. On the other hand, another animal which developed prostration on November 25 from a dose of 3 mg. per kilogram showed no symptoms whatever on November 27 from the same dose.

(c) Multiple sublethal doses: Twelve animals given daily doses of 3 mg. per kilogram of 48/80, intraperitoneally, had marked reactions, with an occasional death. The tendency to marked reactions and to fatalities had not changed at the end of six such injections.

Fourteen animals reacting in less than two minutes to an aerosol of 10 per cent 48/80 (d^2) were given a course of six intraperitoneal injections of 2 mg. per kilogram of 48/80 on the following number of days prior to re-exposure to the aerosol: eight, seven, four, three, two, and one. The reaction time in the aerosol averaged two minutes and thirty-five seconds, and one of the animals died. The average reaction time prior to the intraperitoneal injections was one minute and thirty-five seconds.

2. *Effect of Intraperitoneal Injections of 48/80 on Response to Antigen Aerosol.*—

(a) Single dose: Four guinea pigs passively sensitized with an intravenous injection of 2 ml. of guinea pig antiovalbumin serum were given a 2 mg. per kilogram intraperitoneal injection of 48/80 (d^2) one day later. The following day the animals were exposed to an aerosol of 0.2 per cent crystallized ovalbumin. The end point occurred in two minutes and fifteen seconds on the average. A control group of six sensitized animals which had not received the 48/80 reacted in an average of one minute and forty-five seconds.

(b) Multiple sublethal doses: Another group of eight animals received a course of six injections of 2 mg. per kilogram of 48/80 intraperitoneally on the following number of days before exposure to the aerosol of the antigen; nine, eight, seven, three, two, and one. Two days prior to the challenge they were passively sensitized, as in the previous experiment. The average reaction time of this group to the antigen aerosol was six minutes and fifteen seconds, only one animal reacting in less than four minutes. The control group showed an average of one minute and forty-five seconds.

3. *Effect of Aerosol of 48/80 on Reactivity to Aerosol and Intraperitoneal Injections of 48/80.*—Twenty-six animals were exposed to a 10 per cent aerosol of 48/80 (d^2). Of these, eighteen animals reacted in less than two minutes, one of which died. The seventeen surviving animals were allowed to remain in the chamber a total of three minutes and were then injected intraperitoneally with 3 mg. per kilogram of 48/80. Eight of the fourteen died. The remaining six were exposed two hours later to an aerosol of 10 per cent 48/80. The average reaction time was one minute and thirty-five seconds (about the same as before the intraperitoneal injection of this selected group). Four hours later they were re-exposed to the aerosol, with an average reacting time of one minute and thirty seconds. One of these animals died during the day and one was lost. The remaining four were challenged again in twenty-four hours, with an average reacting time of one minute and fifty-seven seconds.

4. *Effect of Compound 48/80 on Localization of Trypan Blue.*—It was thought that the intensity of the bluing of the skin might be used as a basis of determining histamine release and depletion. The ventral surface of eight albino guinea pigs was depilated with a barium sulfate paste. Three of the animals had previously had three daily sublethal peritoneal injections of compound 48/80 and it was presumed that they had a partial depletion of histamine. The five control animals, as well as those previously treated with compound 48/80, were given an intravenous injection of 5 per cent trypan blue, varying from 1.3 to 2.6 ml. per kilogram. All animals showed marked bluing of the ears, feet, and conjunctiva. The control animals were then given five intracutaneous injections of 0.05 ml. amounts of histamine, varying from concentrations of 1:8,100,000 to 1:100,000. On the opposite site a series of seven intradermal injections was made with compound 48/80 in concentrations ranging from 1:8,100,000 to 1:11,000. The intracutaneous injections did not disclose any increased

color. Since no increased bluing was obtained with histamine or compound 48/80 in the control animals, the "depleted" animals were not tested as there would be no basis for comparison.

The possibility that the general bluing in the previous experiment might have been too great to enable the perception of any additionally induced local changes led us to repeat the experiment. Two animals receiving 0.5 ml. per kilogram of 5 per cent trypan blue had no perceptible bluing. The intracutaneous injection of histamine (1:900,000) or compound 48/80 (1:33,000) failed to blue the local area.

5. *Effect of an Antihistamine on Compound 48/80 Reaction.*—Twelve guinea pigs were given Phenergan, 5 mg. per kilogram, intraperitoneally. One hour later these animals received intraperitoneal injections of compound 48/80 ("a" and "d") 3 mg. per kilogram. Nine of the animals died. Of eighteen control animals receiving the same dose of compound 48/80 without pretreatment with Phenergan, twelve died. In another experiment using doses of 10 mg. per kilogram of compound 48/80 in which the control mortality was 12/12, a prior dose of 10 mg. per kilogram of Phenergan diminished the mortality rate of the histamine liberator to 9/12. The autopsies showed contracted hemorrhagic lungs and a congested liver.

The effect of an intraperitoneal injection (10 mg. per kilogram) of Phenergan on the response to an aerosol from 10 per cent compound 48/80 was then observed. Three animals showed an average end point of three minutes and seven seconds, while three animals that did not receive Phenergan reacted in an average time of two minutes and thirty seconds. An injection of Pyribenzamine, 2 mg. per kilogram, gave results with the compound 48/80 aerosol similar to those of the controls. Three animals exposed to 2 per cent Pyribenzamine aerosol for ten minutes and then immediately exposed to the aerosol of compound 48/80 failed to show any significant protection.

DISCUSSION

When histamine is administered to the guinea pig systemically or by aerosol and when an antigen is administered in like fashion to the sensitized animal, asthma and distension of the lungs are the most prominent features of the response. When compound 48/80 was administered in lethal or near lethal doses there were no typical asthmatic symptoms. The acute effects were scratching, weakness, and prostration, and at times some embarrassment in respiration. An autopsy showed that the lung was contracted and hemorrhagic. When compound 48/80 was administered by aerosol to the point of convulsions and suffocation, the lungs still failed to show the findings typical of acute asthma and emphysema. While there is evidence presented by others that in vitro and in perfusion experiments guinea pig lung tissue releases histamine, it would appear that in the living animal either very little histamine is liberated from the lung by this compound or other toxic actions of the drug kill the animal before sufficient histamine can be released from that organ. The marked scratching, however, does lead one to suspect that histamine is released from the skin.

Repeated daily intraperitoneal injections of compound 48/80 failed to make the animals refractory to further action of the drug. In other words, the fatal dose in the animals injected the first time was the same as in those which had received a number of preceding injections. Neither was the reaction time to the aerosol of compound 48/80 shortened by previous administration of intraperitoneal or aerosol doses. It would appear logical to suppose that if the toxicity of the drug in guinea pigs remains about the same after repeated administration, the toxic effect is not due to histamine release and depletion. These experiments corroborate the findings of Feldberg and Mongar[12] that very little histamine is released by the guinea pig lung perfused with compound 48/80.

The reduced reactivity to the antigen aerosol after repeated intraperitoneal injections of compound 48/80 needs an explanation other than histamine depletion. It is possible that here the action of compound 48/80 is to produce a "stress" similar to those we have previously described from nonspecific injections, which results in partial inhibition of asthma induced by antigen aerosols.[21]

The limited effects of injected or aerosolized compound 48/80 in the inhibition of toxic or fatal reactions to this substance also support our other findings. Doses of antihistamines several times larger than those required to protect against histamine or anaphylactic shock have failed to prevent effectively fatal reactions in the guinea pig from compound 48/80.

The preceding experiments would indicate that in the living guinea pig compound 48/80 releases little histamine from the lungs. Our attempts failed to determine by means of dye localization whether skin areas repeatedly injected with this compound might deplete skin histamine. Trypan blue injected intravenously was not concentrated in the skin areas injected with compound 48/80. Perhaps this dye differs in behavior from Pontamine Sky Blue previously used for somewhat similar experiments.

SUMMARY

1. As shown by the previous results of other workers, compound 48/80 is a potent histamine liberator and depletor in many animals, in vivo and in vitro.

2. In the guinea pig the injection or inhalation of the compound fails to produce asthma or emphysema as is commonly seen in anaphylactic or histamine intoxication.

3. The symptoms in the guinea pig are itching of the skin, prostration, convulsions, and sometimes embarrassed respiration.

4. Autopsy findings show a hemorrhagic, contracted lung and a congested liver.

5. Repeated administration of this compound intraperitoneally or by aerosol fails to make the animals refractory to this drug.

6. The concept that release of histamine is the major toxic action of this drug is weakened by the poor inhibiting effects of antihistamines.

7. These findings do not detract from the establishment of this compound as a substance which releases or depletes histamine in other animals, or in the

guinea pig under different conditions. It must be concluded, however, that in the living guinea pig other toxic effects, at present undetermined, predominate. These toxic effects present an obstacle to the use of this compound as a histamine liberator and depletor.

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Editorial

SECOND INTERNATIONAL CONGRESS ON ALLERGOLOGY

NOTICES concerning the Second International Congress on Allergology, to be held in the Quintandinha Hotel, Rio de Janeiro, Brazil, Nov. 6 to 13, 1955, have already been published in this JOURNAL. As the time approaches when those who are to attend the meeting must make definite plans, it seems opportune to call the attention of all those interested in allergy to the need of supporting this Congress. The International Association of Allergology, founded at the Congress in Zurich in 1951 can be expected to continue only if it is supported by physicians throughout the world who manifest their interest by attending the Congress.

From even casual perusal of the medical literature dealing with allergy, it is apparent that important fundamental work is being done in widely scattered centers throughout the world. It is equally obvious that the diffusion and integration of studies from different laboratories are accomplished far better by personal contact and free discussion than by the printed word. Those who attended the Congress in Zurich were impressed with the contribution that such large international meetings can make to the progress of allergy.

Plans for the scientific sessions include both general sessions and twelve to twenty sectional meetings, each covering one aspect of allergic disease. At the general sessions the guest speakers will include such well-known authorities as Sir Henry Dale, Dr. Pasteur Vallery-Radot, and Dr. Robert A. Cooke. The many sectional meetings will provide ample opportunity for any physician attending to present his own observations and to join in the discussions. It is anticipated that facilities for immediate translation of papers into all the principal languages will be available. Those desiring to present papers should communicate with Dr. Fred Wittich, President of the International Association of Allergology and Chairman of the Program Committee for the Congress.

In these times of tension and unrest, those attending the Congress also may feel that, by their cooperation, they are contributing to international amity and good will as well as to medical progress. The social aspects of the Congress are being carefully planned by the local committee and those who attend are assured of a memorable visit. It is hoped that all who can will evidence their support by being present.

American Academy of Allergy

REPORT OF THE COMMITTEE OF THE AMERICAN ACADEMY OF ALLERGY ON THE ACCEPTABILITY OF ALLERGIC DONORS IN THE AMERICAN RED CROSS BLOOD PROGRAM

THIS Committee was appointed by the president with the approval of the Executive Committee of the American Academy of Allergy to study a series of problems outlined in a letter from Dr. Sam P. Gibson, Associate Director of the American Red Cross Blood Program, Washington, D. C.

The Committee approached the assignment by compiling and studying a series of relevant questions, including those raised in Dr. Gibson's letter. A list of recommendations regarding the acceptability of allergic donors in the American Red Cross Blood Program was then formulated.

Transfusion reactions due to causes other than allergy have not been considered. The term *allergy,* for the purposes of this report, has been limited to such common manifestations as hay fever and allergic rhinitis, asthma, urticaria, angioneurotic edema, atopic dermatitis, and drug and serum sickness. Hypersensitivity to bacteria and contact dermatitis have been excluded from consideration. The emphasis in this report has been placed on allergic reactions to stored or processed bloods and to blood fractions.

1. *What Is the Incidence of Allergic Reactions to Transfusions?*—The literature does not provide a satisfactory answer. Lundy and associates[7] reported an incidence of 2.52 per cent allergic reactions in 7,957 transfusions. Levinson[13] encountered only four allergic reactions (0.22 per cent) in 1,800 transfusions. Other estimates[4, 5, 6, 8] varied from 1 to 4 per cent. The criteria by which these reactions were judged to be allergic were not given. Most of the reactions were stated to be mild and transient.

2. *What Type of Allergic Reactions May Result From Transfusions?*—Immediate or prompt reactions may occur during or shortly after transfusions and are likely to include the most severe reactions. Immediate reactions result from the interaction of antigens, injected with the blood or present in the recipient, and sensitizing antibody, present in the recipient or injected with the blood. Such reactions may take the form of typical or modified attacks of urticaria (pruritus, erythema, whealing, edema), allergic rhinitis (sneezing, rhinorrhea, itching mucous membranes), asthma (cough, dyspnea, wheezing), or other allergic manifestations.

Delayed reactions which occur hours, days, or weeks after transfusions are most likely to be the result of passive sensitization of the recipient by sensitizing antibodies from the donor. The recipient reacts to the subsequent injection, ingestion, or inhalation of the antigen, specifically related to the transfused sensitizing antibody, with an allergic reaction.

3. *Should Antihistamines Be Added Routinely to Bottles of Blood to Prevent or Diminish Immediate Allergic Reactions in the Recipient?*—The Committee advises against it, in spite of recommendations to add them by Ferris, Alpert, and Coakley[4] and by Frankel and Weidner.[5]

Objections to adding antihistamines to the bottles of blood are based on the following considerations:

A. Prior oral administration to the recipient should serve the same purpose as the intravenous administration, with less risk.

B. The recipient may be sensitive to the antihistamine. This is not uncommon. Allergic reactions to drugs given intravenously are likely to be severe.

C. Antihistamines are potential sensitizers.

D. Pretreatment of the patient with an antihistamine should increase its effectiveness. The explosive types of allergic reaction are likely to occur before the transfusion is completed, that is, before the entire dose of the drug in the bottle has had time to run in and become effective.

E. Multiple transfusions containing antihistamines may result in drug overdosage.

F. The routine addition of antihistamines to the bottles of blood might give the physician a false sense of security.

G. Antihistamines would not prevent delayed reactions.

H. The use of antihistamines should be the prerogative of the physician who is responsible for the transfusion.

4. *Is the Giving of Blood by an Allergic Person Harmful to the Donor?*—There is no evidence to indicate that the effect of blood donation on an allergic donor is different from that on a nonallergic person of comparable physical condition.

5. *May Packed Red Cells From an Allergic Donor Be Used Safely?*—Yes. It has been shown[9] that red blood cells from allergic donors, washed twice with Tyrode's solution, do not sensitize normal skin passively.

6. *What Is the Incidence of Sensitization of Recipients by Blood From Allergic Donors?*—Relatively few such cases have been reported in the literature. Tumpeer and Rubens[1] and Loveless[2] provide bibliographies. In the reported cases the donors were found to have exceptionally high titers of sensitizing antibody.

The recipient of such blood may become passively sensitized to the antigen to which the donor was sensitive. In such cases the recipient may, at a subsequent date, develop allergic symptoms when he inhales, ingests, or is

injected with that antigen. This type of sensitization is rarely seen and, when it does occur, lasts only a few days to a few weeks, and usually results in relatively mild reactions which may be readily controlled by simple anti-allergic medication.

The recipient of blood containing sensitizing antibody may develop an immediate or prompt reaction if he ingests, inhales, or is injected with significant quantities of the related antigen shortly before, during, or after the transfusion.

7. *How Long Will Sensitizing Antibodies Persist in Plasma or Serum at Room Temperature?*—Sensitizing antibodies are only slightly impaired after several weeks at room or incubator temperatures. They are not affected by long periods at icebox or freezing temperatures. They may be weakened or destroyed by heating at 56° C. for thirty minutes or more, by the addition of preservatives, by repeated Seitz filtration, or by similar procedures.

8. *May Blood From Allergic Donors Be Used in the Albumin, Gamma Globulin, and Plasma Processing Programs?—*

A. *Albumin:* Theoretically, there are no sensitizing antibodies in pure albumin. However, human blood albumin, as prepared for the Red Cross, contains 1 to 5 per cent of alpha globulin, a potential source of small amounts of sensitizing antibody.[3] Suitable dilution by pooling, discussed in C below, should remove the risk of using blood from allergic donors in the albumin program.

B. *Gamma globulin:* Vaughan, Favour, and Jaffee[3] found small amounts of sensitizing antibody in gamma globulin fractions obtained by Cohn's method No. 10. They cited similar results with other methods.[10] Most of the sensitizing antibodies, however, have been found in the alpha and beta globulin fractions. Hamman[11] found no allergic reactions in 5,000 recipients of gamma globulin. The available evidence suggests that the risk of using blood from allergic donors in the gamma globulin program is negligible, particularly since this fraction is prepared from pooled bloods and only small amounts are generally used for therapeutic injection.

C. *Whole plasma:* The routine processing of whole plasma should have little effect on sensitizing antibodies unless preservatives are used or the specimens are passed repeatedly through Seitz or similar filters.

Tumpeer and Rubens[1] reported few sensitizing antibodies in pooled convalescent serum, and implied that the dilution of highly potent sensitizing serum in a pool of normal plasma seemed to vitiate its capacity to sensitize, although similar dilutions of the same serum with saline did not. This work has not been confirmed and, because of its possible significance in relation to the blood and plasma programs, should be restudied.

Sera containing sensitizing antibodies potent enough to sensitize the skin passively in a saline dilution of 1 to 100 are relatively uncommon. Hence, in most instances, pooling plasma from an allergic donor with nine or more plasmas

lacking the same sensitizing antibodies should eliminate the risk of passive sensitization because the recipient's blood would further dilute the antibodies at least ten times more.

In estimating the likelihood of inducing passive sensitization by transfusion, it is worthy of note that among adult patients who complain of hives, food or drug sensitivities, or perennial allergic rhinitis and asthma, more than one-half have little or no demonstrable sensitizing antibodies in their bloods. The highest incidence of sensitizing antibodies occurs among pollen-sensitive patients with seasonal hay fever or asthma. Hence, the available evidence seems to justify the use of blood from allergic donors in the plasma program if the allergic blood is pooled with ten or more nonallergic bloods, or even with allergic bloods lacking the same sensitizing antibody.

9. *Are Allergic Recipients More Likely to Develop Allergic Transfusion Reactions Than Normal Recipients?*—Yes. The Committee is of the opinion that the allergic state of the recipient is probably the most important factor in the problem of allergic transfusion reactions. Maunsell[12] reported a higher incidence of allergic reactions among allergic recipients than among normal recipients.

10. *What Circumstances Favor the Occurrence of Allergic Transfusion Reactions?*—The following represent at least three situations in which allergic reactions from transfusion are likely to occur:

A. The transfusion of sensitizing antibodies into nonallergic or allergic recipients who have recently ingested, inhaled, or been injected with the allergen specifically related to the sensitizing antibody.

B. The transfusion of foods, inhalants, or drugs in the donor's blood into a recipient sensitive to those allergens. This applies equally to allergic and nonallergic donors.

C. The transfusion of blood or plasma containing both sensitizing antibody and its related antigen. This type of reaction, although theoretically possible, has not been reported. Such reactions could occur from blood taken from an allergic patient while he is having active symptoms or shortly after he has received an injection of the antigen to which he is sensitive. There is also the possibility that reactions may develop from blood donated during the incubation period of the serum sickness type of reaction to drugs, antibiotics, or serum. This suggests the advisability of deferring the bleeding of candidates who have received drugs or injections of allergens for an arbitrary period of two weeks. The indications for this precaution are clearer in connection with direct transfusion and the plasma program than with the albumin and gamma globulin programs.

RECOMMENDATIONS

1. Subject to certain limitations, blood from allergic donors who are symptom-free when bled may be safely accepted for assignment to any one of the following four Red Cross programs:

A. Washed packed red blood cells.

B. Albumin processed from pooled plasma.

C. Gamma globulin processed from pooled plasma.

D. Whole plasma, if pooled with 100 nonallergic plasmas or with plasmas lacking in the same antibodies. It is probably safe to use plasma from allergic donors if it is pooled with only ten nonallergic plasmas.

2. Antihistamine drugs should not be added to bottles of blood or plasma routinely. The prophylactic use of antihistamine drugs should be the prerogative of the physician responsible for the transfusion. Oral pretreatment with antihistamines is preferable when feasible.

3. To avoid the introduction of potent food allergens into allergic recipients, it would be preferable to have all bleedings made from fasting donors, or from those who have partaken of foods which are rarely allergenic. A standard list of such foods may be provided.

4. It would be preferable for donors to refrain from taking any medication for at least forty-eight hours before bleeding.

5. It would be preferable to defer for about two weeks the bleeding of donors who have received drugs, antibiotics, or sera which are known to produce drug or serum sickness type reactions or who have received injections of allergens such as dust, pollen, or mold extracts.

6. The Red Cross should, if possible, arrange for the systematic investigation of allergic reactions to transfusions by competent workers in an effort to identify the mechanisms involved.

7. Because many of the preceding recommendations, in the absence of clinical data, are based solely on theoretical considerations, the Red Cross should implement the study of the effectiveness of processing and pooling in the albumin, globulin, and plasma programs (1) on the elimination of sensitizing antibodies from the sera of allergic donors, and (2) on the removal of allergens from donated blood.

Although the problems of direct transfusions are fundamental and important, they are omitted from this report. It is the opinion of the Committee that, because of the complexity of this subject, an enlarged Committee consisting of an equal number of transfusionists and allergists should study this aspect of transfusion reactions.

In this report transfusion reactions due to nonallergic causes have not been considered.

The Committee will be glad to consult further on these or related problems.

Respectfully submitted,
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Approved by the Executive Committee of
The American Academy of Allergy

JOHN M. SHELDON, President

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Announcements

Second International Congress of Allergology

The Second International Congress of Allergology will be held, by invitation of the Brazilian Allergy Society, in Rio de Janeiro, Nov. 6 to 13, 1955, under the chairmanship of Dr. F. W. Wittich, Minneapolis, Minnesota, President of the International Association of Allergology. A wide and extensive program dealing with almost all important problems of allergy and related immunology, biochemestry, pharmacology, and therapeutics has been elaborated, and is under preparation by the I. A. A. and the Brazilian Allergy Society.

Sir Henry H. Dale of London, Professor Pasteur Vallery-Radot of Paris, Professor Bernardo Houssay of Buenos Aires, and Dr. Robert Cooke of New York City have been invited as guest speakers. The official languages will be English, French, and Spanish, and the main topics will be presented in these three languages, although scientific communications and discussions may be presented in the native language of the author.

The scope of the program is wide, including symposia on:

Histamine, Its Metabolism and Its Role in Allergy.
Histamine Liberators.
Drug Allergy.
Allergic and Toxic Reactions to Drugs.
Haematologic Responses to Drugs.
Cross Sensitizations.
Chemistry of Antigenes and Antibodies.
Cellular Antibodies.
Quantitative Immunological Techniques Applied to Allergy.
Localization of Antibodies.
Physiopathology of the Asthmatic Patient.
Electrolytic Disturbances in Asthmatic Patient.
Death Due to Asthma.
Status Asthmaticus.
Climatic Asthma.
Hormonotherapy of Asthma.
Hormones and Allergy.
Allergy to Homologous Hormones.
Antihistamine Substances.
Specific and Nonspecific Treatments in Allergy.
Psychosomatic Aspects of Allergy.
Dermatologic Allergy.
Industrial and Contact Dermatitis.
Allergy to Animal Parasites.
Allergy to Microbial Organisms.

A limited number and selection of scientific communications will be presented.

A very interesting program, including scientific and technical exhibits, receptions, excursions, entertainments, banquets, and also a ladies program elaborated by the Brazilian Allergy Society, will make this Congress a never-to-be-forgotten meeting.

All information concerning the Congress is available from the General Secretary of the I. A. A.:

Dr. Bernard N. Halpern
197 Boulevard Saint Germain
Paris (7º), France

or from the Secretary of the Sociedade Brasileira de Alergia:

Dr. Fabiano Alves
Avenida Rio Branco 277
7º andar, sala 7 05
Rio de Janeiro, Brazil.

Canadian Academy of Allergy

The Canadian Academy of Allergy will hold its annual meeting on Tuesday, June 21, 1955, at the Royal York Hotel in Toronto, Canada. This meeting will be held during the course of the conjoint meeting between the British Medical Association, Canadian Medical Association, and Ontario Medical Association. The guest speakers at the Academy meeting will be Dr. David A. Long of the National Research Council, London, England, who also will speak at the general session, and Dr. Robert A. Cooke, Director of the Institute of Allergy, Roosevelt Hospital, New York.

A full day of papers, both clinical and laboratory, is planned, and included on the program are such leading Canadians in the field of allergy as Dr. Bram Rose of Montreal and Dr. C. H. A. Walton of Winnipeg.

It is hoped that this program will attract the attention of all those who practice medicine, for it will be of interest to everyone and not just to those who practice allergy.